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# VIOLENCE

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VOL. 18, SEC. C. DECEMBER, 1940 NUMBER 12


## WINTER GROWTH IN THE VEGETATIVE BUDS OF THE WAGENER APPLE[1]


BY HUGH P. BELL[2]



### Abstract

Vegetative buds of the apple were collected from September 26, 1938, to March 18, 1939. The median longitudinal section of each bud was measured. The data collected suggested that a slow but continuous growth in length within the bud proceeds throughout the winter months. The figures were subjected to statistical analysis and found to be significant.


### Introduction

A study of the vegetative bud of the apple throughout a number of winters, suggested that a slight increase in axis length took place even during the coldest months (2, p. 348). The work reported below was undertaken to obtain more detailed and accurate information on this subject.

The literature is not very helpful. There are many references to the vital activities during dormancy of seeds, but very few to dormancy of vegetative buds. In fact, no recent article could be located that gave definite information regarding growth in size within the vegetative bud during the winter. The standard textbooks on plant physiology are usually noncommittal on the subject, but some make definite statements and these are not always in agreement; for instance, Jost (4, p. 345) states: "According to Askenasy's (1877) researches a *complete rest*, where growth is at an absolute standstill, does not take place in the buds. In the flower-buds of the cherry a continuous but feeble *embryonic* growth goes on from summer till next spring." Askenasy (1, p. 804) studied the flower-buds of the cherry and what he actually says is: "The development of the flower-buds of the cherry is divided into two periods which are separated by a period of rest or very small growth." In apparent disagreement with Jost's statement, Maximov (6, p. 290) deals with winter activity within the bud as follows: "In general, all vital activities manifest themselves during dormancy with the exception of growth, which does not take place, though the external conditions may be quite favourable for it." In view of the above, a short statement regarding the data obtained on this subject should be of value.


[1] *Manuscript received July 25, 1940.*

*Contribution from the Department of Biology, Dalhousie University, Halifax, N.S., with financial assistance from the National Research Council of Canada.*



[2] *Professor of Botany.*

## Materials and Methods

The material for this investigation was obtained from a normal Wagener tree at the Dominion Experimental Farm, Kentville, Nova Scotia. Buds, collected twice each month from September 26, 1938, to March 18, 1939, were killed in chrom-acetic. In the selection of individual buds great care was taken to make each one a "random selection", and moreover each collection included buds from all sides and parts of the tree and from different parts of the branch. This material should be typical for winter buds in Nova Scotia, for the meteorological reports indicate that the winter of 1938–39 was an average season. As an example, the winter temperature records from September, 1935, to March, 1940, are given in Table I.

TABLE I

WINTER TEMPERATURES AT KENTVILLE, NOVA SCOTIA, DEGREES F. SEPTEMBER, 1935 TO MARCH, 1940

| | Max. | Min. | Mean |
|---|---|---|---|
| Sept. 1935 | 83 | 33 | 55.81 |
| Oct. 1935 | 73 | 24 | 47.10 |
| Nov. 1935 | 68 | 23 | 42.22 |
| Dec. 1935 | 43 | −9 | 25.49 |
| Jan. 1936 | 50 | −5 | 22.29 |
| Feb. 1936 | 47 | −4 | 17.54 |
| Mar. 1936 | 68 | 4 | 38.79 |
| Sept. 1936 | 78 | 32 | 56.75 |
| Oct. 1936 | 73 | 21 | 47.18 |
| Nov. 1936 | 68 | 7 | 35.5 |
| Dec. 1936 | 57 | 0 | 27.76 |
| Jan. 1937 | 55 | 0 | 26.37 |
| Feb. 1937 | 46 | −8 | 25.6 |
| Mar. 1937 | 46 | 8 | 28.4 |
| Sept. 1937 | 89 | 34 | 59.04 |
| Oct. 1937 | 71 | 26 | 47.4 |
| Nov. 1937 | 59 | 13 | 38.17 |
| Dec. 1937 | 50 | −7 | 26.87 |
| Jan. 1938 | 57 | −13 | 21.26 |
| Feb. 1938 | 42 | −11 | 17.73 |
| Mar. 1938 | 57 | −15 | 25.52 |
| Sept. 1938 | 82 | 33 | 57.80 |
| Oct. 1938 | 78 | 27 | 49.34 |
| Nov. 1938 | 70 | 11 | 39.78 |
| Dec. 1938 | 59 | 2 | 28.57 |
| Jan. 1939 | 45 | 1 | 21.02 |
| Feb. 1939 | 58 | −7 | 21.54 |
| Mar. 1939 | 50 | 1 | 24.57 |
| Sept. 1939 | 89 | 29.5 | 58.56 |
| Oct. 1939 | 78 | 20 | 48.94 |
| Nov. 1939 | 56 | 16 | 34.22 |
| Dec. 1939 | 59 | 7 | 27.20 |
| Jan. 1940 | 35 | −6 | 16.12 |
| Feb. 1940 | 46 | −10 | 20.67 |
| Mar. 1940 | 57 | −5 | 28.57 |

It is difficult to estimate growth in length within a bud, for when preparing material for imbedding in paraffin, it is necessary to remove the outer scales and young leaves. Hence there is no "bench mark" or "base line" from which to make measurements. To overcome this difficulty, the buds were not dissected, and the basal portion of the petiole of the most distal leaf was left attached. The buds were then imbedded in celloidin and cut so that the median longitudinal section passed through the base of this most distal petiole. This median section was projected on drawing paper at a magnification of 53 and the outline was sketched. On each sketch a line was drawn from the centre of the crown down through the central axis of the bud, and a perpendicular was dropped on this line from the axil of the leaf, that is, from the point where the ventral surface of the petiole joined the stem just below the terminal bud. The distance along the median line from the centre of the crown to the point of juncture with the perpendicular, was taken as the *height of the bud axis*, and the distance along the perpendicular from the axil of the leaf to the central axis line, was taken as the *radius of the bud base*. These two dimensions were measured on the outline of each median section.

## Results and Discussion

Owing to the great variation in size of the buds in each collection, it appeared that the most reasonable way to test for increase in height of the bud axis was to study the ratio of height to radius. A typical example of the measurements for one collection is given in Table II. The averages of the measurements and their ratios, for each collection, are given in Table III. A graphic representation of the ratios is given in Fig. 1. It will be seen that there is some indication of an increase in height during the winter months, provided that it can be assumed that the radius for an individual bud does not change materially during that same period. The measurements obtained (Table III) appear to justify this assumption.

Mr. John S. Leefe of the Dominion Experimental Farm, Kentville, N.S., and Dr. Donald Mainland, Professor of Anatomy, Dalhousie University,

TABLE II

MEASUREMENTS OF THE BUDS COLLECTED ON OCTOBER 3, 1938. THE ACTUAL SIZE OF THE BUD IS MAGNIFIED BY 53

| Bud No. | Height of bud axis, cm. | Radius of bud base, cm. | Ratio of height of bud axis to radius of bud base |
|---|---|---|---|
| 1 | 7.60 | 6.95 | 1.09 |
| 2 | 7.15 | 6.65 | 1.07 |
| 3 | 7.40 | 7.15 | 1.03 |
| 4 | 6.45 | 5.75 | 1.12 |
| 5 | 8.05 | 7.20 | 1.11 |
| 6 | 7.56 | 7.77 | 0.97 |
| 7 | 9.56 | 6.46 | 1.48 |
| 8 | 6.90 | 7.20 | 0.95 |
| 9 | 6.25 | 6.60 | 0.94 |
| 10 | 7.40 | 7.25 | 1.02 |
| Averages | 7.43 | 6.79 | 1.07 |

TABLE III

THE AVERAGE OF MEASUREMENTS AND THE RATIO FOR EACH COLLECTION. THE ACTUAL SIZE IS MAGNIFIED BY 53

| Date of collection | Average height of bud axis, cm. | Average radius of bud base, cm. | Average of ratios of height of bud axis to radius of bud base | No. buds measured |
|---|---|---|---|---|
| 26–9–38 | 5.68 | 6.01 | 0.94 | 9 |
| 3–10–38 | 7.43 | 6.79 | 1.07 | 10 |
| 17–10–38 | 6.86 | 6.47 | 1.05 | 10 |
| 31–10–38 | 6.87 | 6.20 | 1.07 | 11 |
| 14–11–38 | 8.08 | 6.94 | 1.16 | 10 |
| 28–11–38 | 7.32 | 6.34 | 1.15 | 11 |
| 12–12–38 | 7.02 | 6.42 | 1.08 | 10 |
| 27–12–38 | 6.44 | 5.95 | 1.08 | 11 |
| 9–1–39 | 7.37 | 6.63 | 1.11 | 10 |
| 24–1–39 | 6.74 | 5.63 | 1.07 | 9 |
| 6–2–39 | 8.10 | 6.80 | 1.19 | 11 |
| 24–2–39 | 7.22 | 6.16 | 1.17 | 11 |
| 6–3–39 | 7.05 | 6.31 | 1.16 | 10 |
| 18–3–39 | 7.61 | 6.28 | 1.30 | 11 |

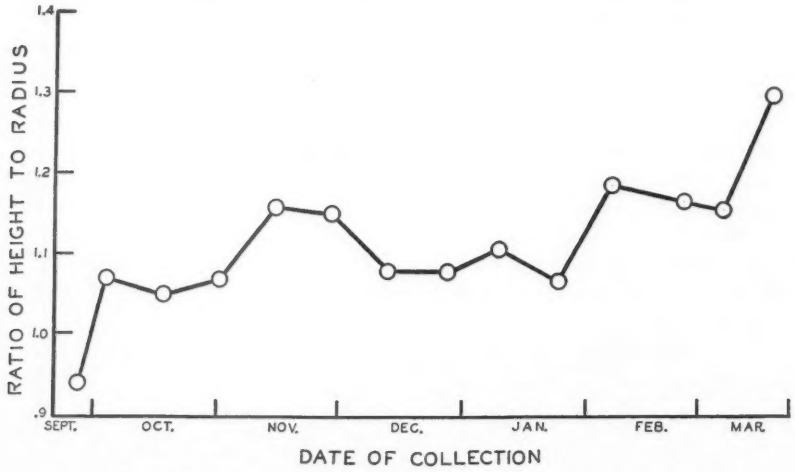


FIG. 1. *Variation in the ratio of height of bud axis to radius of bud base during the period of investigation. (Table III, Columns 1 and 4.)*

Halifax, N.S., have been kind enough to submit the data illustrated in Fig. 1 to statistical analysis, and the writer is indebted to Dr. Mainland for the following statement:

"Since no claim to generalization is based on this series of observations from a single tree in one winter, it does not seem desirable to give many numerical details of the statistical tests to which the data were subjected,

but rather to mention the methods, those prescribed by Fisher, (3), and the results of the tests.

"The data had been prepared as ratios of bud height to bud radius; but ratios are notoriously liable to introduce fallacies (5, p. 269), and in this instance a narrowing of the buds, i.e., a diminution of radius, would produce an increase in the ratio without increase in height. This possibility may not be botanically likely, but it was desirable to prove its absence. As would be expected, the taller buds had, on the average, the longer radii (coefficient of correlation (+0.59). There was no tendency for the radii to change within the period surveyed (correlation coefficient, −0.07), but the method of partial regression showed that, for any given radius, there was a statistically significant upward trend in the corresponding bud height. The partial correlation coefficient representing this relationship between height and time, with radius eliminated, was +0.26, and the regression coefficient, representing rate of increase in height, was 0.069 cm. per unit of time (about two weeks) in terms of the magnified bud sizes, i.e., actually about 13 $\mu$.

"From the original height : radius ratios, a linear regression equation was derived, to represent the straight line that best fitted the graph in Fig. 1. Analysis of variance was carried out by the method of Fisher (3, sect. 44), the general principles of which are briefly described by Mainland (5, p. 280). and the following facts were demonstrated:

"(1) The slope of the regression line was significant, i.e., the increase in the ratios, represented by Fig. 1, could have occurred by chance less than once in 1000 times.

"(2) The increase in the ratio with lapse of time was adequately represented by the straight regression line, i.e., there was no reason to suppose that the downward slopes in the line of the ratios in Fig. 1 represent a real cessation of the main upward trend.

"(3) The average increase in the ratio per unit time interval of about two weeks was 0.0128.

"As just stated, there is no adequate reason to believe that the upward trend in bud size was other than uniform, i.e., the irregularities in Fig. 1 are not significant, but the graph suggests that future investigations be directed to the central period, namely the months of December and January."

In view of Dr. Mainland's statement, it can be concluded that there is a slow growth in the vegetative bud of the apple throughout the winter months, and that this growth produces an increase in length of the bud axis. About the December-January irregularity in the curve of Fig. 1 nothing can be stated with certainty until more observations have been made.

## Acknowledgment

The author wishes to express his indebtedness to the staff of the Laboratory of Plant Pathology, Kentville, Nova Scotia, for making the collections of the buds during the winter of 1938–39.

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# VEGETATIVE PROPAGATION OF CONIFERS

## VIII. EFFECTS OF MEDIA AND PHYTOHORMONE DUST TREATMENTS ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By N. H. Grace[2] and J. L. Farrar[3]


### Abstract

Dormant Norway spruce cuttings collected in November were treated with talc dusts containing indolylacetic acid, planted in five media including two different sands and mixtures of these with peat humus, and propagated in a greenhouse. While 1000 p.p.m. indolylacetic acid treatment increased survival in sand and there were interactions between media and phytohormone treatments, the marked differences between the various media were the striking feature of the results. Mixtures of peat humus with sand were uniformly superior to sand only. There were also some differences between a fine and a coarse sand, when used either alone or in combination with peat. It may accordingly be concluded that selection of a suitable medium is of great importance in the propagation of Norway spruce cuttings.


It has been shown that the rooting response of Norway spruce cuttings depends on a number of factors (2–12). The two most important considerations observed to date relate to the stage of growth at which the cuttings are taken and to the media in which propagation occurs. While treatment with root growth stimulating chemical has resulted frequently in injurious effects, when these chemicals are applied by the carrier dust method some marked beneficial effects have been noted (7, 9). The most impressive results from use of these chemicals were obtained on treatment of dormant cuttings which were subsequently propagated in a mixture of sand and peat in equal proportions (9). Outside propagation in such a medium gave results superior to those obtained in sand only. The combination of certain media and chemical treatments had marked stimulatory effects on new growth as well as on root development. In view of these findings a greenhouse experiment has been carried out with various dust treatments used in conjunction with a series of five media. The results of this experiment are outlined in this communication.

### Experimental

Norway spruce branches were collected November 8, 1939, from the lower region of the tree. The plantation was 19 years of age and situated at the Petawawa Forest Experiment Station, Chalk River, Ontario. In order to ensure a supply of cuttings representative of the entire plantation, not more than two branches were taken from any one tree. The branches were stored outside, covered loosely with straw, and cuttings prepared December 29.

[1] *Manuscript received July 20, 1940.*

*Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, and the Dominion Forest Service, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 957.*

[2] *Biochemist, National Research Laboratories.*

[3] *Assistant in Forestry and Laboratory Research, Dominion Forest Service, Ottawa.*

The cuttings ranged in length from 6 to not more than 12 cm. and were plain, without a heel of old wood. There were from five to six lateral cuttings to one terminal.

The experiment was carried out in a propagation frame which contained 60 glazed earthenware crocks, each provided with drainage. This experimental arrangement has been described in an earlier communication (5). The chief advantage of this arrangement was the isolation of the cuttings in each of the 60 crocks, preventing any contamination from cross diffusion and permitting the use of a number of different media. The propagation frame was provided with a factory cotton screen to reduce light intensity and maintain the humidity, and was situated in a greenhouse room whose temperature approximated 65° F. for the duration of the experiment.

The experiment was of factorial design and comprised four dust and five media treatments, giving 20 different combinations or treatment groups. The four dust treatments involved groups of 15 cuttings without treatment, with talc only, and with 1000 and 3000 p.p.m. (parts of chemical by weight to 1,000,000 parts of the talc mixture) of indolylacetic acid in talc. These four treatment groups were planted in fine sand, coarse sand, one-half of fine sand and one-half peat humus, one-half coarse sand and one-half peat humus, and, finally, three-quarters of coarse sand with one-quarter of peat humus (9). There were three replicate blocks of the 20 treatments. Within each block there were five replicates of the dust or phytohormone treatments, and four replicates of the media treatments. Each of the 60 crocks contained one group of 15 cuttings. The entire experiment required 900 cuttings.

TABLE I

SCREEN ANALYSIS OF SANDS USED AS MEDIA

| Mesh, openings to the inch | Fine sand | | Coarse sand | |
|---|---|---|---|---|
| | Retained, % | Passed, % | Retained, % | Passed, % |
| 10 | 2.1 | 97.9 | 6.8 | 93.2 |
| 14 | 4.2 | 95.8 | 14.7 | 85.3 |
| 28 | 35.5 | 64.5 | 51.3 | 48.7 |
| 35 | 72.3 | 27.7 | 73.4 | 26.6 |
| 48 | 92.1 | 7.9 | 88.6 | 11.4 |
| 65 | 97.1 | 3.0 | 94.2 | 5.8 |
| 100 | 99.1 | 0.9 | 97.1 | 2.9 |
| 200 | >99.9 | <0.1 | 99.3 | 0.7 |

Brown builder's sand was obtained from two local pits and one sand apparently was considerably finer than the other. The results of screen analyses of these two sands are given in Table I. It is apparent that the "coarse" sand has more of the very large and very small fractions than the "fine" sand. The material designated as coarse sand actually forms a denser mass and has approximately 10% less pore space than the fine.

Cuttings were dusted and planted to a depth of about one inch as soon as possible, in no case more than two hours after preparation. It might be pointed out that the cuttings were not moistened prior to dusting, though they were kept covered with a moist cloth for the short period between preparation and dusting. Ottawa tap water was used for watering throughout the course of the experiment.

Fifteen weeks after planting, the cuttings were removed for examination. Record was made of the number of cuttings surviving, rooted, callused, with new growth, and with new growth and rooted. The number and length of roots were determined and from the resulting data were calculated the number and length of roots per rooted cutting and the mean root length. The number and lengths of all new growth shoots were determined. These data permitted calculation of the length of new growth per cutting with new growth, and length of terminal new growth per cutting with terminal new growth. Data on numbers of cuttings were subjected to the inverse sine transformation prior to analysis of variance (1). All the data were subjected to analyses of variance.

Living non-rooted cuttings were replanted in the respective media after the first examination and taken up for a final count eight weeks later.

## Results

In Table II are given results of the analyses of variance. Part A of the table deals with the number of surviving cuttings and refers to the results in all five media. Owing to exceedingly poor rooting in sand with meagre data not suited to the analysis of variance procedure, analyses of the other observations were restricted to the three media containing peat-sand. These results are given in Part B of the table. Significant effects from media or chemical treatment were to be noted in 8 of the 10 sets of observations considered. In all eight the effect of media was significant—with one exception, highly significant. There were significant interactions between media and chemical treatments with respect to the number of surviving cuttings, the mean length of all new growth shoots and the length of terminal new growth.

Data for the effects of media and dust treatments on the number of surviving cuttings are given in Table III. The effects of media, averaged over all dust treatments, were such that all peat mixtures showed a greater number of surviving cuttings than sand only. Fine sand was below coarse in respect to number of surviving cuttings, but the mixture of peat with fine sand was superior to its combination with the coarse. The results indicated that the combination of one-quarter peat with three-quarters sand was not so good a medium as equal proportions of peat and sand. The interaction of media with phytohormone treatments was such that whereas the 1000 p.p.m. concentration of indolylacetic acid increased survival in both sand media, the higher concentration indicated a falling off in the beneficial effect of the chemical; these effects, however, were not observed in the peat-sand mixtures, which gave high survival rates irrespective of treatment. Talc alone suggested some beneficial effect in sand but statistical significance was not obtained.

TABLE II

ANALYSIS OF VARIANCE OF RESPONSES TO MEDIA AND PHYTOHORMONE DUST TREATMENTS OF NORWAY SPRUCE CUTTINGS

| Source of variance | Degrees of freedom (A)—surviving cuttings only | Mean square—number of surviving cuttings | Degrees of freedom (B)—observations in three media with peat | Mean square | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number of rooted and callused cuttings | number of rooted cuttings | Number of roots per rooted cutting | Length of root per rooted cutting | Mean root length | Number of cuttings with new growth | Number of rooted cuttings with new growth | Mean length of all new growth shoots | Mean length of terminal new growth shoots |
| Replicates | 2 | 187.2 | 2 | 256.2* | 98.5 | 0.03 | 141.5 | 12.6 | 357.4 | 128.2 | 8.78 | 9.37 |
| Treatments | 3 | 143.3 | 3 | 159.1 | 146.4 | 0.40 | 151.9 | 24.1 | 63.1 | 88.2 | 1.66 | 8.19 |
| Media | 4 | 3097.4*** | 2 | 168.5 | 843.3*** | 0.61 | 3996.9** | 351.0* | 1491.1*** | 1034.4*** | 245.20*** | 459.70*** |
| Interaction treatments × media | 12 | 159.8* | 6 | 73.2 | 81.5 | 0.12 | 605.1 | 92.6 | 59.7 | 49.1 | 20.16* | 15.77* |
| Error | 38 | 73.7 | 22 | 67.7 | 69.2 | 0.28 | 537.3 | 64.5 | 134.9 | 75.2 | 7.57 | 5.51 |

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

## TABLE III

EFFECTS OF MEDIA AND PHYTOHORMONE DUST TREATMENTS ON THE NUMBER OF SURVIVING CUTTINGS

| Treatment | Kind of data | Medium | | | | |
|---|---|---|---|---|---|---|
| | | Sand | | Peat humus with | | |
| | | Fine | Coarse | One-half fine sand | One-half coarse sand | Three-quarters coarse sand |
| Untreated | Transformed | 27.1 | 35.0 | 69.6 | 74.0 | 62.2 |
| | Per cent | 22.2 | 33.3 | 86.7 | 88.9 | 77.8 |
| Talc | Transformed | 36.5 | 48.2 | 70.8 | 77.9 | 60.9 |
| | Per cent | 35.6 | 55.6 | 84.5 | 93.3 | 75.6 |
| 1000 p.p.m. indolylacetic acid in talc | Transformed | 49.5 | 56.3 | 67.5 | 62.7 | 65.8 |
| | Per cent | 57.8 | 68.9 | 84.5 | 77.8 | 82.2 |
| 3000 p.p.m. indolylacetic acid in talc | Transformed | 26.3 | 43.1 | 75.7 | 66.9 | 65.1 |
| | Per cent | 20.0 | 46.7 | 91.1 | 84.5 | 82.2 |
| Means, media treatments | Transformed | 34.9 | 45.7 | 78.4 | 70.4 | 63.5 |

*Necessary difference, 5% level between means of media treatments, 7.2; for interaction, 14.4.*

## TABLE IV

AVERAGE EFFECTS OF MIXTURES OF PEAT AND SAND ON THE RESPONSES OF NORWAY SPRUCE CUTTINGS

| Response | Peat humus with | | | Necessary difference, 5% level |
|---|---|---|---|---|
| | One-half fine sand | One-half coarse sand | Three-quarters coarse sand | |
| Number of rooted cuttings, transformed data | 44.0 | 37.5 | 27.4 | 7.0 |
| Per cent | 48.3 | 37.2 | 23.3 | |
| Number of roots per rooted cutting | 2.7 | 2.6 | 2.4 | |
| Length of roots per rooted cutting, mm. | 64.4 | 39.6 | 28.9 | 19.6 |
| Mean root length, mm. | 22.8 | 15.2 | 12.2 | 8.1 |
| Number of cuttings with new growth, transformed data | 64.1 | 57.1 | 42.3 | 9.8 |
| Per cent | 78.9 | 68.9 | 45.6 | |
| Number of rooted cuttings with new growth, transformed data | 41.7 | 35.1 | 23.4 | 7.3 |
| Per cent | 44.4 | 33.3 | 17.8 | |
| Mean length of all new growth shoots, mm. | 25.3 | 19.5 | 16.3 | 2.3 |
| Mean length of terminal new growth shoots, mm. | 28.1 | 21.0 | 15.8 | 2.0 |

In Table IV are given data for the average effects of mixture of sand with peat humus on various other responses of the cuttings. Although several of the observed differences are statistically insignificant, it would seem that the mixture of equal proportions of peat with fine sand is to be preferred to the corresponding mixture with coarse sand. The poorest results were obtained with the medium low in peat. In several instances the differences ascribable to media were large in magnitude. Several of the responses indicated somewhat greater differences ascribable to the two proportions of peat rather than to the texture of the sand. A notable exception to this tendency was indicated by the length of roots per rooted cutting, substantially greater root length occurring in the fine sand. Although the mixtures of peat with the two sands did not differ significantly in respect to the number of cuttings with new growth, the combination with fine sand was much the best in so far as the mean length of all new growth shoots was concerned. However, the data on lengths of new growth shoots should be considered in detail in relation to the various dust treatments, as a significant interaction of these factors has been indicated in Table II.

TABLE V

MEAN LENGTH OF ALL NEW GROWTH SHOOTS, MM.

| Medium | Untreated cuttings | Cuttings treated with talc containing indolylacetic acid, p.p.m. | | |
|---|---|---|---|---|
| | | 0 | 1000 | 3000 |
| 50% peat humus, 50% fine sand | 26.7 | 23.7 | 22.7 | 28.0 |
| 50% peat humus, 50% coarse sand | 17.3 | 19.7 | 19.7 | 21.3 |
| 25% peat humus, 75% coarse sand | 17.7 | 17.7 | 17.0 | 13.0 |

*Necessary difference, 5% level, 4.6 mm.*

Interaction effects of phytohormone dust treatments and media are indicated in the data of Table V, for the mean length of new growth shoots. In the mixture of peat and fine sand none of the treatments resulted in a mean length of new growth significantly different from that produced by the untreated cuttings. In the mixture of equal proportions of peat and coarse sand, shoot length tended to increase with phytohormone dosage, whereas in the mixture of 75% coarse sand and 25% peat this tendency was significantly reversed.

Similar data for the mean length of terminal new growth shoots are given in Table VI. The effects of phytohormone treatment were essentially the same as just described for mean shoot length in Table V. However, in the mixture of coarse sand and one-quarter peat, talc only resulted in a length of terminal new growth significantly greater than that shown by the untreated cuttings. Further, the highest level of indolylacetic acid did not reduce the length of terminal growth in any of the media.

TABLE VI

MEAN LENGTH OF TERMINAL NEW GROWTH SHOOTS, MM.

| Medium | Untreated cuttings | Cuttings treated with talc containing indolylacetic acid, p.p.m. | | |
|---|---|---|---|---|
| | | 0 | 1000 | 3000 |
| 50% peat humus, 50% fine sand | 28.7 | 25.3 | 26.7 | 31.7 |
| 50% peat humus, 50% coarse sand | 20.0 | 20.0 | 21.0 | 23.0 |
| 25% peat humus, 75% coarse sand | 14.0 | 18.0 | 16.7 | 14.3 |

*Necessary difference, 5% level, 3.9 mm.*

Whereas the first examination had indicated that 36.3% of the total number of cuttings planted had rooted, the final count eight weeks later raised this to 43.5%. Thus, during the eight weeks subsequent to the first count an additional 7.2% of the cuttings rooted. However, statistical analysis of these final counts still failed to demonstrate any effects of chemical treatment on the number surviving. Media effects were again of the same order as at the first examination.

## Discussion

The effects of media on the rooting responses of Norway spruce cuttings are the outstanding feature of these results. While meagre rooting in the two sands rendered consideration of complete data for this part of the experiment impossible, the results in the three peat mixtures indicate that under the conditions of this trial, the composition of the media had a much greater effect than had phytohormone dust treatments. The number of surviving cuttings was greater in coarse than in fine sand. However, in combination with peat the fine sand effected uniformly superior results. Differences attributable to the sand were most marked in measurements on root length and new growth. The amount of peat in the mixture appeared to have greater effect than the kind of sand on the number of rooted cuttings and the number rooted with new growth. These media differences would appear to be related to such factors as aeration, water holding capacity, or available essential chemical elements (5, 8, 9).

Although there were several interactions between dust treatments and media, phytohormone treatments failed to have marked effects.

These effects from mixtures of peat and sand are in general agreement with the results from the outside propagation of a November collection of cuttings (9), although the latter also indicated marked beneficial effects from phytohormone treatment. In contrast, earlier greenhouse experiments with a mixture of sand and an imported granulated peat moss failed to indicate significant improvement from this medium. Indeed, some injurious effects

were noted (7, 8). Increased survival in sand on treatment with 1000 p.p.m. indolylacetic acid was in agreement with the results of an earlier experiment (7).

It may be observed that the low rooting in sand only was rather surprising in the light of previous results under similar greenhouse conditions (7). There was, however, one difference in the propagation conditions in this and the former experiments. In the former the sand was watered heavily. In the present, watering was reduced and the sand maintained moist rather than thoroughly drenched. The results suggest that the moisture-sand relations recommended by propagators for many plants are not sufficiently moist for the successful propagation of Norway spruce cuttings in sand.

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# MENDELIAN INHERITANCE OF CERTAIN PATHOGENIC CHARACTERS OF *PUCCINIA GRAMINIS TRITICI*[1]

By Thorvaldur Johnson[2] and Margaret Newton[3]

## Abstract

Crossing and selfing studies with physiologic races of *Puccinia graminis Tritici* have shown that certain pathogenic characters are dominant to others. The "0" type of infection (absence of rust pustules) on the variety Kanred was found to be dominant to the "4" type (large rust pustules), so that when a race producing the "0" type was crossed with a race producing the "4" type the hybrid rust produced the "0" type. When the hybrid race was selfed, the "0" type occurred about three times as frequently in $F_2$ as the "4" type, a fact indicating that rust behaviour on this variety is governed by a single-factor pair. The "4" type of infection on the variety Mindum normally was dominant to the "1" type (very small pustules) and occurred about three times as frequently in $F_2$. The "1" type of infection on the emmer variety Vernal was dominant to the "4" type and recurred in some crosses, about 15 times as frequently in $F_2$ as the "4" type. Rust behaviour on this variety appears to be governed by duplicate factors, each factor being capable of exerting the same effect. Evidence derived from a study of the $F_2$ populations of two crosses between races 9 and 36 indicated that the factors governing rust behaviour on Kanred, Mindum, and Vernal, were different and were inherited independently of each other. In crosses in which the two parent races produced different infection types on the variety Marquis, the cytoplasm of the maternal parent race appeared to influence pathogenicity on this variety.

As a result of these studies it is concluded that despite the binucleate condition of stem rust in its uredial phase, the genes function as if they were present in a single diploid nucleus, and that, owing to fusion of the nuclei in the teliospore and subsequent meiotic divisions, independent segregation of factors occurs as in higher plants. The crossing of physiologic races and the selfing of the hybrids lead to various recombinations of existing pathogenic characters that may result in the formation of new physiologic races without involving the creation of pathogenic characters not possessed by the parent races.

## Introduction

The identification of physiologic races of the cereal rusts is based on the fact that these races produce definite infection characteristics (infection types) on selected cereal host varieties. In *Puccinia graminis Tritici* Erikss. and Henn., identification of physiologic races is conducted by determining the infection types produced on 12 wheat varieties. Six rather well defined infection types designated as 0, 1, 2, 3, 4, and x occur on these varieties. These infection types, originally described by Stakman and Levine (9) are illustrated in Fig. 1. Because these infection types are relatively constant under ordinary greenhouse conditions, they may be regarded as characters of the rust organism and may, like other characters of living organisms, be subjected to inheritance studies when crosses are made between two physiologic races with sharply contrasted infection types on a given host plant. A study of the infection types produced on the variety Kanred (or Reliance) may serve as an example. On this variety many physiologic races produce either

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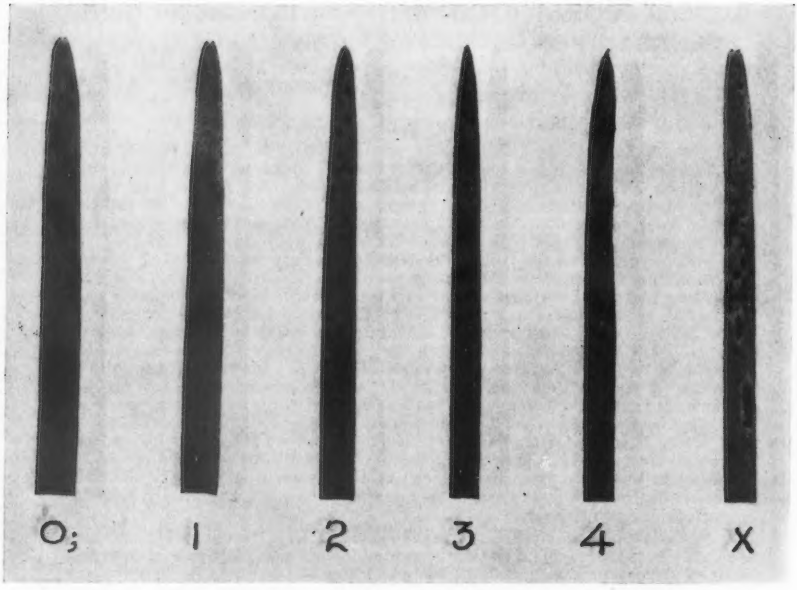


FIG. 1. *Six infection types caused by physiologic races of Puccinia graminis Tritici on differential wheat varieties.*

no visible signs of infection or, at most, minute necrotic flecks. This is known as a "0" type of infection. Other races produce large pustules: a "4" type of infection. If a race which is homozygous for the "0" infection type is crossed with a race homozygous for the "4" infection type, it is easy to demonstrate the dominance or recessiveness of this pair of contrasted characters by inoculating leaves of the variety Kanred with spores of the $F_1$ hybrid of the cross. If the $F_1$ hybrid race produces the "0" type of infection, it is clear that this type is dominant to the "4" type. Should a study of the inheritance of these characters in the $F_2$ generation be desired, the hybrid rust may be selfed in order to determine the frequency distribution of the two infection types in that generation. The same method of study may be applied to the infection types produced on any other host variety. In the study here reported, attention has been given chiefly to the inheritance of infection characteristics on the varieties Marquis, Kanred, Mindum, and Vernal. The methods used in crossing and selfing studies have been described in previous publications (5, 7). It is the purpose of the present paper to record and interpret the results of the crosses that have thus far been made between physiologic races of *Puccinia graminis Tritici*. Preliminary accounts of this work have appeared in earlier papers (4, 6).

## Dominance of Certain Characters in $F_1$

The crosses and their $F_1$ progeny are recorded in Table I which also includes comments on the inheritance of those pathogenic characteristics by which the two parent races differed. Particular attention was given to the inheritance of the "0" and "4" infection types on Kanred, the "1" and "4" types on Mindum, and the "1" and "4" types on Vernal. The choice of these infection types was largely decided by the fact that several races were available that were homozygous for these characters.

In the crosses between races 9 and 36, and 9 and 52, reported in earlier papers (4, 5, 8), it was noted that, for Kanred, the "0" infection type produced by race 9 was dominant to the "4" type produced by races 36 and 52; that, for Mindum, the "4" type produced by race 9 was dominant to the "1" type produced by the other races; whereas, for Vernal, the "1" type produced by race 36 was dominant to the "4" type produced by race 9. These results are illustrated below by reference to a cross between races 9 and 36.

| | | Kanred | Mindum | Vernal |
|---|---|---|---|---|
| Parents | Race 9 | 0 | 4 | 4 |
| | Race 36 | 4 | 1 | 1 |
| $F_1$ | Race 17 | 0 | 4 | 1 |

These findings raised the question whether these results are peculiar to crosses between the above-mentioned races or whether they are of general occurrence in crosses between races of wheat stem rust. The crosses recorded in Table I were made largely with the purpose of throwing light on this question.

Not all the races used in these crosses were homozygous for their infection types on all three varieties. A race homozygous for its infection type on Kanred might be heterozygous for its infection type on Mindum or *vice versa*. The conclusions derived from any cross have reference, chiefly, to the characters for which the parent races were homozygous.

*Infection Types on Kanred.*—In all except one of the 49 crosses between races homozygous for the "0" and "4" types of infection on Kanred, the "0" type proved dominant, and in all crosses in which both parent races produced a "4" type of infection on this variety the hybrid race also produced the same infection type. No crosses were made between two races both of which produced the "0" type. As far as can be judged from the crosses that have been made, it appears that the dominance of the Kanred "0" type is a common phenomenon in stem rust.

*Infection Types on Mindum.*—In crosses between races producing "1" and "4" types of infection on Mindum, the "4" type has been commonly, though not invariably, dominant. In the 31 crosses between races homozygous for

TABLE I

CROSSES BETWEEN PHYSIOLOGIC RACES OF *P. graminis Tritici*

| Races crossed | Varieties on which parent races are homozygous for contrasted infection types | No. of crosses | Races in $F_1$ | Comments on pathogenicity in $F_1$ |
|---|---|---|---|---|
| 9* × 36 | Krd, Mnd, Ver | 6 | 17 | Krd "0" dominant to Krd "4" |
| 36 × 9 | | 3 | 17 | Mnd "4" dominant to Mnd "1"<br>Ver "1" dominant to Ver "4" |
| 9 × 15 | Krd | 1 | 9 | Krd "0" dominant to Krd "4" |
| 15 × 9 | | 1 | 9 | |
| 9 × 52 | Krd, Mnd | 1 | 9 | Krd "0" dominant to Krd "4" |
| 52 × 9 | | 2 | 9 | Mnd "4" dominant to Mnd "1" |
| 52 × 57 | Krd | 8 | 57 | Krd "0" dominant to Krd "4" |
| 52 × 120 | Krd | 2 | 57 | Krd "0" dominant to Krd "4" |
| 52 × 15 | Mnd | 4 | 15 | Mnd "4" dominant to Mnd "1" |
| 1 × 15 | Krd, Mnd | 2 | 9 | Krd "0" dominant to Krd "4" |
| 1 × 15 | | 1 | 17 | Mnd "4" dominant to Mnd "1" |
| 57 × 36 | Krd, Ver | 5 | 1 | Krd "0" dominant to Krd "4"<br>Ver "1" dominant to Ver "4" |
| 36 × 36 | | 1 | 36 | |
| 36 × 21 | Krd | 4 | 17 | Krd "0" dominant to Krd "4"<br>Mnd "4" dominant to Mnd "1" |
| 36 × 15 | Mnd, Ver | 4 | 11 | Mnd "4" dominant to Mnd "1" |
| 36 × 15 | | 1 | 110 | Ver "4" dominant in four crosses but infection type intermediate in one |
| 36 × 120 | Krd, Ver | 1 | 155 | Krd "0" dominant in 12 of 13 crosses |
| 36 × 120 | | 5 | 136 | Ver "1" dominant in 8 of 13 crosses; infection type intermediate in five crosses |
| 36 × 120 | | 3 | 157 | Mnd "1" appeared in 11 crosses, Mnd "4" in two |
| 36 × 120 | | 1 | 158 | |
| 36 × 120 | | 1 | 172 | |
| 120 × 36 | | 1 | 136 | |
| 120 × 36 | | 1 | 158 | |
| 125 × 11 | Mnd | 1 | 11 → | Mnd "4" dominant to Mnd "1" |
| 125 × 11 | | 1 | 126 → | Infection type on Mnd intermediate |
| 36 × 51 | Ma? Ver? | 1 | 175 | Ma "2" dominant to Ma "4" |
| 51 × 36 | | 1 | 152 | Ver "1" dominant to Ver "4" in one cross but infection type intermediate in the other |

TABLE I—*Concluded*

CROSSES BETWEEN PHYSIOLOGIC RACES OF *P. graminis Tritici*—*Concluded*

| Races crossed | Varieties on which parent races are homozygous for contrasted infection types | No. of crosses | Races in $F_1$ | Comments on pathogenicity in $F_1$ |
|---|---|---|---|---|
| 36 × 14 | Ma, Mnd | 4 | 88 | |
| 14 × 36 | | 1 | 14 | |
| 36 × 152 | Ma | 3 | 171 | |
| 152 × 36 | | 3 | 152 | |
| 39 × 125 | Ma | 2 | 173 | In all these crosses, one parent race produces a "1" or "2" type of infection on Marquis, the other a "4" type. In the hybrid progeny, infections on Marquis resemble those of the maternal parent in each cross. |
| 39 × 125 | | 1 | 174 | |
| 39 × 125 | | 3 | 177 | |
| 39 × 125 | | 1 | 39 | |
| 125 × 39 | | 3 | 18 | |
| 125 × 39 | | 2 | 56 | |
| 125 × 39 | | 1 | 125 | |
| 125 × 39 | | 1 | 34 | |

* *In all the crosses, the parent race placed first is the race on whose haploid pustules aecia were produced as a result of a transfer to them of the pycniospore-containing nectar of the other race.*

these characteristics, 30 produced the "4" infection type and one the indeterminate "x" type. In the 22 crosses in which both parent races produced the "1" type of infection, the $F_1$ hybrids also produced that infection type. Only in crosses of which one parent race was heterozygous for its infection type on Mindum did both infection types appear in the $F_1$ generation.

*Infection Types on Vernal.*—In the majority of crosses between races homozygous for the "1" and "4" infection types on Vernal, the "1" type proved dominant. In 34 such crosses, 27 gave rise to the "1" type while 7 produced an "x" (indeterminate) infection type. The "1" and "4" types of infection, both, appeared only in the $F_1$ progeny of crosses in which one parent race was heterozygous for the "1" type of infection, as in the cross 1 × 15 where race 1 was heterozygous. All the 19 crosses between races producing the "4" type on Vernal gave rise to $F_1$ progeny with the "4" infection type. Eleven crosses between races homozygous for the "1" type of infection produced only progeny with that infection type (36 × 36, 36 × 21, 36 × 152). The rather frequent appearance of the "x" type of infection in crosses between races homozygous for the "1" and "4" types seems to show that dominance of the "1" type is sometimes incomplete.

*Infection Types on Marquis.*—Crosses of races producing a "1" or "2" type of infection on Marquis with races producing a "4" type on this variety bring

TABLE II

INFECTION TYPES PRODUCED ON DIFFERENTIAL WHEAT VARIETIES BY THE PHYSIOLOGIC RACES OF *P. graminis Tritici* USED IN OR DERIVED FROM CROSSING AND SELFING STUDIES

| Race | L.C. | Ma. | Krd. | Ko. | Arn. | Mnd. | Spm. | Kub. | Ac. | Enk. | Ver. | Kpl. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 4− | 0 | 3+ | 1= | 1 | 1= | 3+ | 3++ | 3 | 0; | 1= |
| 9 | 4 | 4− | 0 | 3++ | 4− | 4= | 4= | 4= | 3++ | 3+ | 4± | 1− |
| 11 | 4− | 4= | 3++ | 3+ | 4= | 4= | 4= | 3++ | 3++ | 3 | 1= | 1= |
| 14 | 4+ | 2− | 1− | 1++ | 3++ | 3++ | 3++ | 3++ | 3++ | 3 | 1= | 0; |
| 15 | 4 | 4− | 4= | 3++ | 4= | 4= | 4= | 3++ | 3++ | 3++ | 4± | 1= |
| 17 | 4 | 4− | 0 | 3+ | 4= | 4= | 4= | 3++ | 3++ | 3 | 1= | 1= |
| 18 | 4 | 4− | 4= | 3++ | 1 | 1= | 1− | 3++ | 3++ | 3+ | 1− | 1± |
| 21 | 4 | 4 | 0 | 3++ | 4− | 4− | 4− | 4= | 3++ | 1= | 0; | 1= |
| 29 | 4 | 4− | 0 | 3 | x++ | x± | x+ | x | x+ | 3 | 1− | 1− |
| 32 | 4 | 4= | 4= | 3+ | x+ | x± | x± | x− | x+ | 3 | 1= | 1− |
| 34 | 4+ | 4− | 4− | 4= | 4 | 4= | 4= | 4± | 3++ | 1= | 0; | 1± |
| 36 | 4 | 4 | 4− | 3++ | 1= | 1= | 0; | x | 3++ | 3+ | 0; | 1− |
| 39 | 4− | 2= | 4= | 3+ | 4+ | 3++ | 4− | 4= | 3++ | 4= | 1= | 1− |
| 51 | 4 | 2= | 3+ | 0; | 0; | 0; | 0; | 4 | 3++ | 3+ | 4− | 0; |
| 52 | 4 | 4 | 4− | 4 | 1= | 1= | 1= | x± | 4 | 4− | 4+ | 1− |
| 56 | 4 | 3+ | 3+ | 3+ | 1= | 1= | 1= | 3+ | 3+ | 1= | 1= | 1− |
| 57 | 4 | 4− | 0 | 3+ | 1 | 1 | 1 | 4 | 3+ | 3 | 3 | 1 |
| 85 | 4 | 4− | 0 | 3± | 4− | 4 | 4 | 4 | 4 | 3+ | x | 1 |
| 88 | 4 | x | 0 | 1± | 4 | 4 | 4 | 4 | 4 | 3+ | 1 | 1 |
| 110 | 4 | 4− | 3 | 3− | 3+ | 3+ | 3+ | 3+ | 3+ | 3 | x− | 1 |
| 120 | 4 | x | 0 | 3= | 4 | 4 | 4 | 4 | 4− | 3+ | 3 | 1− |
| 125 | 4± | 4 | 4 | 4 | 0; | 0; | 0; | x | 4 | 1= | 0; | 1− |
| 126 | 4= | 4= | 3+ | 3+ | x± | x++ | x+ | x− | x | 1± | 1= | 1− |
| 136 | 4 | x | 0 | 3= | 0; | 0; | 0; | 4− | 4 | 4− | 1− | 1 |
| 152 | 4 | 1+ | 3− | 0; | 1 | 0; | 0; | x− | 3+ | 3+ | 0; | 1− |
| 155 | 4 | x | 0 | 3= | 1 | 0; | 0; | x | 4 | 3+ | 0; | 1 |
| 157 | 4 | x | 0 | 3= | 1− | 0; | 0; | 4 | 4 | 3+ | x | 1− |
| 158 | 4 | x | 0 | 3= | 4 | 4− | 4− | 4− | 4− | 3+ | x | 1 |
| 171 | 4 | x± | 3− | 0; | 1 | 0; | 0; | x− | 3+ | 3+ | 0; | 1− |
| 172 | 4 | x | 3− | 2− | 1− | 0; | 0; | 4− | 4 | 3 | 0; | 1− |
| 173 | 4 | 2 | 4− | 3− | 0; | 0; | 0; | 4 | 4− | 1− | 0; | 1 |
| 174 | 4 | 2 | 4− | 3= | 4− | x | x | 4− | 4− | 1− | 0; | 1 |
| 175 | 4 | 2+ | 4 | 1± | 1− | 0; | 0; | x | 4− | 3± | x | 1− |
| 177 | 4 | 2 | 4− | 3− | 1− | 1− | 1− | 4− | 3+ | 3 | 0; | 1− |

to light a kind of inheritance not encountered in the study of the infection characteristics on the other varieties. In 25 of 27 such crosses, the $F_1$ progeny produced on Marquis an infection type closely resembling that of the maternal parent race, that is, the race on whose haploid pustules aecia were formed. The $F_1$ progeny arising from one side of such a cross produced a "1" or "2" type of infection whereas that from the opposite side produced a "4" type or occasionally an "x" type so heavy that it bordered on a "4" type of infection. This behaviour, in crosses, of the infection types on Marquis has been described for the cross race 36 × race 14 in previous papers (4, 5, 6, 8) in which it was attributed to the influence of the cytoplasm of the maternal parent race. Its occurrence in crosses between other races suggests that it may be a rather general phenomenon. A similar type of inheritance has been shown to govern the infection types on Joanette strain in crosses between physiologic races of *Puccinia graminis Avenae* Erikss. and Henn. (3).

## Selfing Studies

*General Survey of Selfing Studies*

Evidence bearing on the dominance or recessiveness of the above-mentioned infection characteristics may be obtained not only from crossing studies but also from the selfing of physiologic races. If, in the selfing of physiologic races from many different sources, it should be found that a certain characteristic, such as the "4" infection type on Kanred, always reproduces itself in the progeny whereas another characteristic, such as the "0" infection type, frequently produces types differing from itself, it is natural to suppose that the former occurs only in a homozygous state while the latter may exist in either a homozygous or heterozygous condition. In this example, the "0" infection type would be assumed to be dominant to the "4" type.

Since hybridization studies with stem rust were first undertaken by the writers, they have selfed 66 cultures comprising 24 physiologic races. Data on the homozygosity or heterozygosity of these cultures for their pathogenic characteristics on the varieties Kanred, Mindum, and Vernal are presented in Table III.

TABLE III

HOMOZYGOSITY OR HETEROZYGOSITY OF 66 SELFED CULTURES OF WHEAT STEM RUST FOR CERTAIN PATHOGENIC CHARACTERS

| Pathogenic character | No. cultures selfed | Number homozygous | Number heterozygous |
|---|---|---|---|
| Kanred "4" | 26 | 25 | 1 |
| Kanred "0" | 40 | 17 | 23 |
| Mindum "1" | 28 | 24 | 4* |
| Mindum "4" | 36 | 13 | 23 |
| Mindum "x" | 2 | 0 | 2 |
| Vernal "4" | 22 | 17 | 5** |
| Vernal "1" | 44 | 21 | 23 |

* *Two of these cultures may have been homozygous.*
** *Three of these cultures may have been homozygous.*

The data supplied in Table III provide support for the hypothesis that the "4" type of infection on Kanred, the "1" type on Mindum and the "4" type on Vernal, are recessive characteristics. Theoretically, all the cultures showing these characteristics should have proved homozygous. Actually, a few showed indications of heterozygosity. The exceptions shown in Table III are probably the result of undetected impurities in the selfed races, accidental introduction of foreign pycniospore-bearing nectar or mutation.

*The Distribution of Pathogenic Characters in $F_2$*

In several of the crosses mentioned in Table I, one or more $F_1$ hybrid cultures have been selfed with the purpose of discovering the distribution in the $F_2$ generation of characters (infection types) that were dominant or reces-

sive in the cross. The most extensive selfing studies made, thus far, have been carried out with two separate crosses between races 9 and 36. These two races differ in their pathogenic characteristics on the variety Kanred, on the *durum* varieties Arnautka, Mindum, Spelmar and Kubanka, and on the emmer variety Vernal (Table II). In these studies, each $F_2$ culture originated from a single aecial cup selected at random from numerous aecial pustules on several barberry plants infected with the $F_1$ hybrid race—race 17. The frequency distribution in $F_2$ of the contrasted infection types on the varieties Kanred, Mindum, and Vernal is shown in Table IV. The significance of the ratios was determined by the Chi-square ($\chi^2$) method according to the formula $\chi^2 = \Sigma\left[\frac{(a-t)^2}{t}\right]$, where $a$ represents the actual and $t$ the theoretical frequencies of the contrasted infection types.

TABLE IV

FREQUENCY DISTRIBUTION IN $F_2$ OF THE CROSS, RACE 9 × RACE 36, OF THE CONTRASTED INFECTION TYPES ON KANRED, MINDUM, AND VERNAL

| Infection type | Cross I | Cross II | Crosses I and II combined |
|---|---|---|---|
| Kanred "0" | 96 | 131 | 227 |
| Kanred "4" | 30 | 68 | 98 |
| Ratio | 3.2 : 1 | 1.9 : 1 | 2.3 : 1 |
| $\chi^2$ | 0.0952 | 8.8715 | 4.6334 |
| $P$ | 0.75 | 0.01 | 0.03 |
| Mindum "4" | 89 | 142 | 231 |
| Mindum "x" | 0 | 11 | 11 |
| Mindum "1" | 37 | 46 | 83 |
| Ratio | 2.4 : 1 | 3.3 : 1 | 2.9 : 1 |
| $\chi^2$ | 1.2804 | 0.3866 | 0.0531 |
| $P$ | 0.25 | 0.52 | 0.80 |
| Vernal "1" | 120 | 187 | 307 |
| Vernal "x" | 4 | 3 | 7 |
| Vernal "4" | 2 | 9 | 11 |
| Ratio | 20 : 1 | 15.6 : 1 | 17 : 1 |
| $\chi^2$ | 0.4874 | 0.0138 | 0.2778 |
| $P$ | 0.49 | 0.90 | 0.58 |

It is clear from a study of Table IV that the infection types that proved dominant in the cross reappeared much more frequently in the $F_2$ generation than the recessive infection types. In calculating the ratios, the "x" type infections, which occurred occasionally on Mindum and Vernal, were grouped together with the "4" type. The ratios thus obtained approximate rather closely to a 3 : 1 for the infections on Kanred and Mindum and to a 15 : 1 ratio for those on Vernal. To test the goodness of fit to these ratios, the values of $\chi^2$ and $P$ were calculated for each cross as well as for the two crosses combined.

Taking the 5% point as a criterion of significance, the $P$ values, with one exception, provide support for the hypothesis that the behaviour of the rust

on Kanred and on Mindum is in each case governed by a single pair of Mendelian factors and that the infection characteristics on Vernal are governed by two pairs of factors (duplicate factors). The exception is the distribution of infection types on Kanred in cross II.

TABLE V

A TEST (ON THE $F_2$ POPULATIONS OF TWO CROSSES BETWEEN RACES 9 AND 36) OF THE GOODNESS OF FIT OF THE DISTRIBUTION OF THE INFECTION TYPES ON KANRED AND VERNAL AND ON MINDUM AND VERNAL TO THEORETICAL RATIOS CALCULATED ON THE ASSUMPTION THAT THE RUST BEHAVIOUR ON KANRED AND MINDUM IS GOVERNED BY SINGLE FACTOR PAIRS AND THAT ON VERNAL BY TWO-FACTOR PAIRS

| Infection types | | Genotypes | | Theor. ratio | Cross I | | Cross II | | Cross I and II combined | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Actual distribution | Theor. distribution | Actual distribution | Theor. distribution | Actual distribution | Theor. distribution |
| Kanred | Vernal | Kanred | Vernal | | | | | | | |
| "0" | "1" | A | C and/or D | 45 | 91 | 88.6 | 121 | 139.9 | 212 | 228.5 |
| "4" | "1" | a | C and/or D | 15 | 29 | 29.5 | 66 | 46.6 | 95 | 76.2 |
| "0" | "x" "4" | A | c and d | 3 | 5 | 5.9 | 10 | 9.3 | 15 | 15.2 |
| "4" | "x" "4" | a | c and d | 1 | 1 | 2.0 | 2 | 3.1 | 3 | 5.1 |
| | | | | | $\chi^2 = 0.711$ $P = 0.87$ | | $\chi^2 = 10.727$ $P = 0.01$–$0.02$ | | $\chi^2 = 6.696$ $P = 0.09$ | |
| Mindum | Vernal | Mindum | Vernal | | | | | | | |
| "x" "4" | "1" | B | C and/or D | 45 | 84 | 88.6 | 145 | 139.9 | 229 | 228.5 |
| "1" | "1" | b | C and/or D | 15 | 36 | 29.5 | 42 | 46.6 | 78 | 76.2 |
| "x" "4" | "x" "4" | B | c and d | 3 | 5 | 5.9 | 8 | 9.3 | 13 | 15.2 |
| "1" | "x" "4" | b | c and d | 1 | 1 | 2.0 | 4 | 3.1 | 5 | 5.1 |
| | | | | | $\chi^2 = 2.309$ $P = 0.5$ | | $\chi^2 = 1.099$ $P = 0.8$ | | $\chi^2 = 0.364$ $P = 0.95$ | |

Further confirmation of this hypothesis and an indication that these factors are inherited independently are obtained from the data presented in Table V. For this table, a calculation was made of the expected frequencies of all possible combinations of infection types on Kanred and Vernal and on Mindum and Vernal, on the assumption that in each case the infection types on the two hosts were inherited independently of each other. As the infection types on Kanred and Mindum appear to be governed by a single-factor pair and those on Vernal by a two-factor pair, the data should conform to a tri-hybrid ratio in which, for example, in a population of 64 $F_2$ cultures, the "0" type on Kanred and the "1" type on Vernal would be associated 45 times, the "4" type on Kanred and the "1" type on Vernal 15 times, the "0" type on Kanred and the "1" type on Vernal three times, and the "4" type on Kanred and the "4" type on Vernal once. The agreement of the theoretical and actual ratios is very close for the Mindum-Vernal infection types in both crosses ($P$ values 0.5 and 0.8) and for the Kanred-Vernal infection types in cross I

($P$ values 0.87) but is poor for Kanred-Vernal in cross II ($P$ value 0.01 to 0.02). In spite of the absence of statistical evidence in this last instance, it seems probable that the pathogenic behaviour of the rust is governed by one-factor pairs for Kanred and Mindum and by a two-factor pair for Vernal and that these factors are assorted independently of each other.

TABLE VI

ACTUAL AND THEORETICAL DISTRIBUTION OF PHYSIOLOGIC RACES IN THE COMBINED $F_2$ GENERATIONS OF TWO CROSSES BETWEEN RACES 9 AND 36, THE THEORETICAL DISTRIBUTION BEING BASED ON THE ASSUMPTION THAT SINGLE-FACTOR PAIRS GOVERN RUST BEHAVIOUR ON THE VARIETIES KANRED AND MINDUM AND THAT TWO PAIRS OF FACTORS GOVERN RUST BEHAVIOUR ON THE VARIETY VERNAL

| Parent Races | $F_1$ | $F_2$ | | | | |
|---|---|---|---|---|---|---|
| | | Genotypes | Race | Theor. distribution | Actual distribution | |
| *Race 9* | | A B C D<br>A B c D<br>A B C d | 17 | 171.4 | 165[1] | |
| Hosts *Krd Mnd Ver* | | a B C D<br>a B c D | 11 | 57.1 | 64[2] | |
| Genotype AA BB ccdd | *Krd Mnd Ver* | a B C d<br>A b C D | | | | $\chi^2=11.374$ |
| Inf. type "0" "4" "4" | Aa Bb CcDd | A b c D | 1 | 57.1 | 47 | $P = 0.13$ |
| *Race 36* | *Race 17* "0" "4" "1" | A b C d<br>a b C D | | | | |
| Hosts *Krd Mnd Ver* | | a b c D<br>a b C d | 36 | 19.0 | 31 | |
| Genotype aa bb CCDD | | A B c d | 9 | 11.4 | 11[3] | |
| | | A b c d | 57 | 3.8 | 4 | |
| Inf. type "4" "1" "1" | | a B c d | 15 | 3.8 | 2[4] | |
| | | a b c d | 52 | 1.3 | 1 | |

[1] *In this group are included six cultures of race 29 which differs from race 17 by producing an "x" infection type on Mindum instead of a "4" type.*

[2] *In this group are included five cultures of race 32 which differs from race 11 by producing an "x" infection type on Mindum instead of a "4" type.*

[3] *In this group are included six cultures of race 85 which differs from race 9 by producing an "x" infection type on Vernal instead of a "4" type.*

[4] *In this group is included one culture of race 110 which differs from race 15 by producing an "x" infection type on Vernal instead of a "4" type.*

To test this theory further a comparison was made of the theoretical distribution of physiologic races in the $F_2$ generation of crosses I and II with the actual distribution of the races. The results are shown in Table VI. This table gives (i) the supposed genetic constitution of the parent races and of the $F_1$ and $F_2$ hybrids, (ii) the theoretical distribution of the races expected on the assumption that dominance is complete, and (iii) the actual distribution. According to the theory outlined above, the various possible tetra-hybrid combinations should give rise to the following races: 1, 9, 11, 15, 17, 36, 52, and 57. Actually, all these races did occur in the $F_2$ population but so did four others, namely, races 29, 32, 85, and 110. All these, however, bear a rather close

resemblance to certain of the expected races, differing only by their tendency to produce an "x" type of infection on certain differential varieties. Thus race 29 is identical with race 17, and race 32 with race 11, except for a tendency to produce an "x" infection type on certain *durum* varieties including Mindum. Similarly races 85 and 110 resemble races 9 and 15 respectively except for a disposition to produce an "x" infection type on Vernal. The "x" infections on the *durum* varieties are perhaps the result of environmental conditions at the time of race identification rather than genotypic differences between cultures, as this type of infection was confined to one group of cultures identified within a period of less than one month. Owing to the resemblance of the races characterized by "x" infections to others identified much more frequently they have, in Table VI, been grouped together with the races to which they bear the closest resemblance.

Granting this modification, the actual distribution of the physiologic races in the $F_2$ population conforms in most instances rather closely to theoretical expectation, as will be seen in Table VI. As a result, however, of race 36 occurring more frequently and race 1 less frequently than expected, the value of $\chi^2$ is rather high and the value of $P$ rather low. The $P$ value of 0.13 is not, however, low enough to exclude the possibility of the correctness of the proposed scheme of inheritance.

## Discussion

Interpretation of the experimental results presented in this paper leads to the conclusion that the pathogenic properties of physiologic races of *Puccinia graminis Tritici* are inherited, for the most part, in accordance with Mendelian laws of inheritance. There is evidence for the phenomena of dominance, recessiveness, and independent segregation of the factors governing rust behaviour. There is evidence that some pathogenic characters are governed by a single-factor pair, others by more than one factor pair. Genotypically, a physiologic race must be considered to contain many genes or factors that in one way or another affect its pathogenicity. Some of these factors appear to govern the behaviour of the rust on certain differential varieties without apparently affecting rust behaviour on other varieties. There is evidence that the single-factor pair governing the behaviour of the rust on the variety Kanred is different from the single-factor pair governing rust behaviour on the variety Mindum, and it is clear, furthermore, that the two pairs of factors governing the infection types on Vernal differ from both. This does not, of course, imply that a given single-factor pair affects only the rust behaviour on a single variety. On the contrary, it is likely that a given factor pair governs rust behaviour on many closely related varieties. In several of the crosses studied it seemed manifest that rust behaviour on the varieties Arnautka, Mindum, and Spelmar was governed by the same factor pair.

It is not to be assumed, as a result of the presence in certain crosses of 3 : 1 ratios for rust behaviour on Kanred and Mindum and a 15 : 1 ratio for rust behaviour on Vernal, that such ratios are of universal occurrence in the selfing

of heterozygous races. When duplicate factors govern rust behaviour, as appears to be the case in Vernal, different lines descended from the same cross may produce different ratios. In the scheme of inheritance outlined in Table VI, $F_2$ lines with genotypes Ccdd and ccDd should, when selfed, produce 3 : 1 ratios while $F_2$ lines with the genotype CcDd should produce 15 : 1 ratios. The few selfing studies carried out with $F_2$ lines do actually suggest the existence of both ratios.

The question of dominance is of interest in view of the dikaryotic condition of the uredial phase of stem rust. That the phenomenon of dominance is expressed in the uredial stage is shown by crossing and selfing studies alike, and this applies equally to urediospore-colour and pathogenicity. As shown elsewhere (2, 4) red, grayish-brown, or orange spore colour is dominant to white. Among pathogenic characters that have been studied, the "0" infection type on Kanred is dominant to the "4" type; the "4" infection type on Mindum is dominant to the "1" type; the "1" type of infection on Vernal is dominant to the "4" type. In oat stem rust, it has been shown (3) that red urediospore colour is dominant to orange, and that certain infection types on the varieties White Tartar and Richland are dominant to others.

These results make it evident that the binucleate condition of the rust organism does not in any way interfere with the expression of dominance. That this fact is not generally realized is shown by the assertion of Dodge (1) that dominance cannot be expressed in a dikaryophyte. In view of the results enumerated above it would seem that the distinction sometimes made between an uninucleate, diploid organism and a dikaryotic one is purely academic. This distinction has no practical basis if allelomorphic genes function in the same manner whether they are both present in a single diploid nucleus or one in each of the two haploid nuclei of a dikaryophyte. Furthermore, there can be no practical reason for not applying the term "hybrid" to a new dikaryotic combination when it is realized that the genes present act is if they were contained in a true diploid nucleus. When it is further realized that the dikaryon fuses to form a true diploid nucleus (in the teliospore) and that this diploid nucleus divides meiotically, it becomes apparent that the analogy with uninucleate organisms is complete except for the fact that the two nuclei of the dikaryophyte do not fuse during a certain phase of the life cycle of the rust.

One curious characteristic of many crosses between the physiologic races of stem rust of wheat and of oats, is the tendency of the $F_1$ hybrids from opposite sides of the same cross to differ in certain pathogenic characters. As the nuclear material in such reciprocal hybrids should be identical, it does not seem possible to explain this phenomenon in terms of gene action. It is possible that the pycniospores brought over from another race lose their scanty amount of cytoplasm by the time they reach the aecial primordium where diploidization takes place, so that the resultant dikaryon contains nuclear material from both parent races but cytoplasm from only one—the maternal race. The cytoplasmic influence (if such it is) is expressed only in

certain pathogenic characteristics but leaves others unaffected. In crosses between physiologic races of wheat stem rust it is manifest if the parent races differ in their infection types on the variety Marquis, and can be detected only through the infection types on this variety. In crosses between races of oat stem rust it may be discerned in the infection types on Joanette strain (3). In these instances, the influence of the cytoplasm apparently overcomes or obscures the effects of nuclear factors.

In view of the fact that rust behaviour on different wheat varieties may be governed by different factors inherited independently, it can be readily understood why many physiologic races may occur in the progeny of a cross between two races. If the two parent races differ pathogenically on four differential varieties, and if the factors governing these differences are inherited independently, it is clear that, under circumstances of complete dominance, 16 phenotypic classes, that is, physiologic races, may be expected to occur in the $F_2$ generation. In this generation, the pathogenic characters of the parent races are recombined in various ways without, however, necessarily involving the creation of new pathogenic characters. It would seem, therefore, that crossing of races does not necessarily change the pathogenic ability of the rust although new combinations of pathogenic characters present in the parents are brought into being.

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# NOTE ON THE PROPAGATION BY CUTTINGS OF WHITE PINE AND WHITE SPRUCE[1]

By J. L. Farrar[2] and N. H. Grace[3]

Throughout the past three years (1938–1940) the authors have been interested in the vegetative propagation of conifers important in forestry. Preliminary work on a number of spruces and pines indicated that Norway spruce (*Picea Abies* (L.) Karst.) was less difficult to root from cuttings than the others. Consequently much intensive work has been done with this species in the expectation that the discovery of a successful method of rooting Norway spruce would lead to a solution of the problem of rooting our important native conifers (2). Experiments based on this work have now led to the rooting of white spruce (*Picea glauca* (Moench) Voss.) and white pine (*Pinus strobus* L.), and this note gives a preliminary account of the methods and the results obtained. A full report will appear in this Journal at a later date.

The cuttings were collected from a large group of 10- to 15-year-old trees, and were from 5 to 10 cm. long, and of the current year's growth. They were planted in outside frames protected by lath and factory cotton shades, the propagation media rested directly on the ground.

The propagation medium proved to be the most important single factor affecting rooting, a mixture of peat and sand being vastly superior to sand alone. Furthermore, a well-decomposed native peat of sedge origin (1, 2) was found to result in much better rooting than the European peat moss of sphagnum origin commonly used in horticulture. It also had a very pronounced effect in stimulating buds to produce new shoots. Of only slightly less importance was the time of collecting the cuttings. Late July was the most favourable period for white spruce, whereas mid-August was most favourable for white pine. Indolylacetic acid mixed with talc and applied to the base of the cuttings did not have any marked effect on rooting.

Under the optimum combination of the conditions mentioned, white spruce was rooted 80 to 90% on the average, with individual groups of 10 cuttings attaining 100%. Likewise white pine was rooted 50 to 60% on the average, with individual groups of ten cuttings attaining 90%.

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# EFFECT OF CROWN GALL FORMATION ON THE CHEMICAL COMPOSITION OF BEETS[1]

By A. C. Neish[2] and Harold Hibbert[3]

## Abstract

Tumours were produced on *Beta vulgaris* (beet) by inoculation with *Phytomonas tumefaciens* (crown gall organism). Analyses were made of tumorous tissue, normal tissue of tumorous beets, and the tissue of healthy beets (not inoculated) dried at 60° C. in a vacuum oven. The constituents determined quantitatively were glucose, fructose, sucrose, starch, cellulose, lignin, pentosans, uronic anhydride (in holocellulose), total uronic anhydride, pectins, ether extract, crude protein, total ash, tannins, free phenolics, methoxyl, and solid matter. The results are discussed with respect to *(a)* the effect of the bacterial stimulus on the physiology of the plant and *(b)* the resulting changes in chemical composition.

The bacteria exert a local stimulus causing the cells to proliferate and form a tumour. Sucrose is converted into cell wall and protoplasm in the tumour cells. This sucrose is obtained from the normal tissue of the root by the tumour cells. This normal tissue is not stimulated by the bacterium to convert sucrose into other materials. Owing to this increased synthetic activity of tumour cells, an infected root contains more solid matter than a non-infected one, but less sucrose. The composition of the cell wall is essentially the same in tumorous and normal tissue. The conversion of sucrose to protein is a major metabolic process in tumours on beet roots. Ethanolysis of beet root indicates that the lignin present is probably of the same general nature as the lignin in woods.

## Introduction

Strohmer and Stift (17) found that the galls of sugar beets had a higher content of ash, protein, and moisture but a lower content of sugar than normal roots.

Levine (11) found that the total weight of infected beets, grown in fertile soil, was greater than the total weight of non-infected beets and also that the galls developed to the greatest extent on the beets that were the best nourished. Brown and Quirk (4) found the total acid content of tumour juice to be greater than that of juice from normal tissue although the pH of the former was more on the alkaline side. They measured the redox potential with a gold electrode and found the tumour juice to be negative with respect to the normal juice. Binet and Magrou (3) determined reduced glutathione in *Pelargonium zonale* inoculated with *P. tumefaciens*. When the galls were four months old they contained 316 to 437 mg. of glutathione per 100 gm. of fresh tissue; the leaves contained 101 to 122 mg., green stems 83 to 142 mg., and the terminal growing point 500 to 1000 mg. per 100 gm. of fresh tissue. Following necrosis of the gall the glutathione disappeared; thus the latter appears to be associated

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with rapid tissue proliferation. Berthelot and Amoureux (2) showed that beet crown galls contained five times as much reduced glutathione, twice as much ascorbic acid, 20% more nitrogen and 20% less ash than normal tissue. They found that differences in total dry matter, sugars, potassium, and phosphorus were slight and irregular.

Townsend (18) analyzed fresh galled beets (after removal of galls) and healthy beets and found that the healthy beets contained 50% more sucrose than the galled beets. Nagy, Riker, and Peterson (12) found that the galls contained more glutathione than did adjacent tissues but not so much as did the growing tips. They found the galls of sugar beets contained 2.9% total nitrogen as compared with 0.9% in normal tissue. Also, the fresh galls contained 42% of non-reducing sugar (sucrose) as compared with 70% in the normal tissue, dry weight basis. The galled tissue of beets was found to have a somewhat higher content of starch, cellulose, pentosan, ash, and ether extract but little difference was found in uronic acid content. They made similar analyses of galls on tomato and raspberry stems and found the galls to be lower in cellulose and pentosans than the contiguous tissue. These plants are not so succulent as the beet root. Extracts from galls (on tomato) rapidly destroyed tyrosine but stem extracts did not. Catalase, oxidase, and peroxidase activity on a wet-weight basis were 160, 130, and 120% higher, respectively, in galls than in normal tissues. The same figures calculated on the basis of total nitrogen are 86, 73, and 57%, respectively.

Klein and Zeise (9) found the catalase activity in crown gall of beets to be greatly increased over that of contiguous tissue. They also found (10) in galls on horse radish that the increase in catalase was paralleled by an increase in peroxidase.

It is interesting to note that Rasnowski (16) observed that lignification of sugar beets was a hereditary property and that a low sugar content was associated with a high degree of lignification.

The present investigation provides further data on the chemical composition of crown galls on beets and the contiguous tissue, also on that of non-infected controls. It is found that by calculating the analytical data on various bases it is possible to draw conclusions concerning the physiology of the tumours.

## Experimental

*Preparation of Material*

Beets of the variety "Extra Early Flat Egyptian" were grown in pots in a greenhouse, using fairly fertile soil. One hundred plants were raised under the same conditions. Sixty of these were inoculated with *Phytomonas tumefaciens* and the other forty kept as controls.

The seeds were soaked overnight in water and then planted in a flat. When the seedlings reached a height of about two and one-half inches and had developed true leaves they were transplanted into 6-in. pots, one to each pot. When the storage organ (root) reached about one-quarter of an inch in diameter the plants were inoculated on one side of the crown by stabbing

several times with a needle previously dipped into a two day old culture of *P. tumefaciens* grown on beef-peptone agar.

The plants were allowed to develop until the storage organs (roots) reached a diameter of $1\frac{1}{2}$ to $2\frac{1}{2}$ in. (the maximum size reached by this variety under these conditions). The tumours were well developed, being almost equal in size to the rest of the storage organ. The beets were then harvested, the tops and branch roots being removed leaving only the storage organ which was then scrubbed in running water with a test-tube brush until free from soil. The tumours were then removed from the infected beets. Since inoculations were made on one side only, the tumours were present on that side as a swelling which could be readily separated by cutting along a line that marked the natural contour of the beet root. The tumorous tissue did not differ markedly from the normal tissue in macroscopic appearance. The tumours were smooth and firm except at the point where the inoculations were made; here they were puckered. There was no sign of rot nor secondary infection in the beets taken for analysis.

The tissue was cut into thin slices about 2 mm. thick and dried in a vacuum oven at 60° C. for three days. The dried tissue was then ground in a porcelain mortar to give a powder fine enough to pass a 60-mesh screen. Fresh and dry weights were recorded. In this way three samples were obtained.

*(a)* Healthy beets (controls),

*(b)* Normal tissue from tumorous beets,

*(c)* Tumorous tissue.

These are composite samples: *(a)* represents 40 individual beets and *(b)* and *(c)* represent 50 individual beets.

Table I shows the fresh and dry weights of these samples.

TABLE I

FRESH AND DRY WEIGHTS OF SAMPLES

| Type of sample | Fresh weight, gm. | Dry weight, gm. | Percentage of dry matter |
|---|---|---|---|
| Healthy beets | 719.0 | 91.9 | 12.78 |
| Normal tissue of tumorous beets | 729.9 | 71.6 | 9.81 |
| Tumorous tissue | 574.2 | 59.6 | 10.38 |

*Analysis of Samples*

The dried samples were analyzed for the following substances by the methods indicated.

1. *Sucrose, fructose, and glucose.* Copper reduction method of van der Plank (15). It was found that inversion of the sucrose with invertase, takadiastase, and dilute hydrochloric acid yielded substantially the same result.

2. *Starch.* Takadiastase method as outlined by Denny (6).

3. *Crude protein.* Kjeldahl nitrogen was multiplied by the factor 6.25. The nitrogen was determined according to the method described by the Association of Official Agricultural Chemists (1, p. 279).

4. *Ether extract.* Method of the Association of Official Agricultural Chemists (1, p. 279).

5. *Total ash.* Method of the Association of Official Agricultural Chemists (1, p. 278).

6. *Tannins.* A special permanganate titration of an aqueous extract before and after adsorption of tannins on kaolin was employed (as described by the Association of Official Agricultural Chemists (1, pp. 155–156) ).

7. *Pectic substances.* By precipitation as calcium pectate (Nanji and Norman (13) ).

8. *Cellulose.* Schorger's method as described by Dorée (8).

9. *Uronic anhydride.* This was calculated from the carbon dioxide given off on boiling with hydrochloric acid (12%) following the method of Dickson, Otterson, and Link (7). It was determined both on total material and on holocellulose.

10. *Holocellulose* was prepared by the method of Van Beckum and Ritter (19). This material consists of cellulose and hemicellulose free from lignin. The comparatively high yield obtained with beet tissue indicates that it may contain the protein as well.

11. *Free phenols* were estimated in the ether extract using the colorimetric method of Deichmann and Scott (5).

12. *Pentosans.* The method of Kullgren and Tyden (Dorée (8, p. 364)). was used with the holocellulose.

13. *Acid lignin* was determined by Schwalbe's method as described by Dorée (8, p. 349).

14. *Ethanolysis of beet root* was found to give oils with a methoxyl content of 11 to 20%, so it was concluded that lignin in beets is not an artifact formed from carbohydrates.

15. *Methoxyl* was determined, by the method of Peniston and Hibbert (14), in *(a)* total tissue, *(b)* the residue after extraction with ether, and *(c)* the residue after extraction with ether and hot 0.5% aqueous ammonium oxalate. The value for (*c*) should represent the methoxyl of the lignin.

The results of these analyses are given in Table II, expressed as percentage of dry weight. From these values and those given in Table I it was possible to calculate the results on a fresh weight basis (Table III) and on the basis of amount of material in an average individual beet root (Table IV), and also to calculate the ratio of the substance in question to the crude protein (Table V). These results are discussed in detail in the next section.

## Discussion

The results in Table II are expressed as percentage of the dry matter. Examination of these results shows that on this basis the tumours have a higher content of crude protein, ether extract, starch, pectins, pentosans, cellulose, lignin, methoxyl, polyuronides (of holocellulose), and total ash than healthy beets. The latter have a considerably higher content of sucrose than the tumours; the differences in reducing sugars are slight. Pectins and total uronic anhydride are present in lowest amount in the normal tissue of tumorous beets but otherwise this tissue is intermediate in composition between healthy beets and tumorous tissue. The presence of tannins and free phenols is doubtful. Most of the methoxyl is present in an insoluble form, presumably as lignin.

TABLE II

COMPOSITION OF NORMAL AND TUMOROUS TISSUE OF BEETS—RESULTS EXPRESSED AS PERCENTAGE OF TOTAL DRY WEIGHT

| Constituent | Healthy beets | Normal tissue of tumorous beets | Tumorous tissue |
|---|---|---|---|
| Total ash | 9.65 | 13.46 | 13.46 |
| Free glucose | 1.00 | 0.82 | 1.18 |
| Free fructose | 0.56 | 1.03 | 0.72 |
| Sucrose | 47.92 | 29.66 | 12.30 |
| Starch | 1.18 | 2.41 | 2.48 |
| Ether extract | 1.56 | 1.87 | 2.57 |
| Crude protein | 10.39 | 12.12 | 20.03 |
| Pectins | 9.69 | 8.00 | 13.15 |
| Uronic anhydride (in holocellulose) | 5.06 | 6.06 | 7.77 |
| Pentosans (in holocellulose) | 1.98 | 2.34 | 2.65 |
| Cellulose | 6.64 | 7.65 | 9.05 |
| Acid lignin | 4.06 | 4.88 | 5.97 |
| *Total of solid matter accounted for* | *99.69* | *90.30* | *91.03* |
| Total uronic anhydride | 12.47 | 11.36 | 12.93 |
| Total methoxyl | 0.813 | 0.920 | 1.264 |
| Methoxyl of material insoluble in anhydrous ether | 0.807 | 0.916 | 1.259 |
| Methoxyl of material insoluble in ether and hot aqueous ammonium oxalate (0.5%) | 0.704 | 0.850 | 1.064 |
| Tannins | Less than 0.1 | Less than 0.1 | Less than 0.1 |
| Free phenols* | | | |

* *Presence doubtful.*

Summation of the results in Table II shows the solid matter accounted for to be:

(*a*) For healthy beets, 99.69%,

(*b*) For normal tissue of tumorous beets, 90.30%,

(*c*) For tumorous tissue, 91.03%.

In making this summation total uronic anhydride and methoxyl are not included since they come from pectins, uronic anhydride (in holocellulose), and lignin, which already have been included.

Thus practically all the dry matter of the healthy beets has been accounted for but only 90% of that of the infected beets.

The variation in the sucrose content is the most marked result. Naturally when one substance varies so widely, some other component or components of the tissue must necessarily show a variation in the opposite direction when expressed as percentage of dry matter. Examination of the results in Table II therefore do not indicate the conditions existing in the living tissue. To interpret these results in the light of chemical changes in composition associated with tumour formation they must be expressed in various ways.

TABLE III

COMPOSITION OF NORMAL AND TUMOROUS TISSUE OF BEETS EXPRESSED AS PERCENTAGE OF TOTAL FRESH WEIGHT

| Constituent | Healthy beets | Normal tissue of tumorous beets | Tumorous tissue |
|---|---|---|---|
| Total ash | 1.23 | 1.32 | 1.35 |
| Free glucose | 0.13 | 0.08 | 0.12 |
| Free fructose | 0.07 | 0.10 | 0.07 |
| Sucrose | 6.12 | 2.91 | 1.28 |
| Starch | 0.15 | 0.24 | 0.26 |
| Ether extract | 0.20 | 0.18 | 0.27 |
| Crude protein | 1.33 | 1.19 | 2.08 |
| Pectins | 1.24 | 0.78 | 1.36 |
| Total uronic anhydride | 1.59 | 1.11 | 1.34 |
| Uronic anhydride (in holocellulose) | 0.65 | 0.59 | 0.81 |
| Pentosans (in holocellulose) | 0.25 | 0.23 | 0.27 |
| Cellulose | 0.85 | 0.75 | 0.94 |
| Acid lignin | 0.52 | 0.48 | 0.62 |
| Total methoxyl | 0.10 | 0.09 | 0.13 |
| Methoxyl insoluble in anhydrous ether | 0.10 | 0.09 | 0.13 |
| Methoxyl insoluble in anhydrous ether and ammonium oxalate (0.5%) | 0.09 | 0.08 | 0.11 |
| Tannins* | | | |
| Free phenols* | | | |

* *Presence doubtful.*

In Table III the results are expressed as percentage of the fresh (undried) weight. Since this material is approximately 90% water it is apparent that comparing the amounts of a substance in equal weights of fresh tissue is essentially the same as comparing the amounts in equal volumes of fresh tissue. Therefore the results in Table III permit comparison of the concentrations of the substances *in the living tissues* of tumorous and healthy beets.

Table IV expresses the results as amount of the substances in an average individual beet root and enables one to obtain a measure of the effect of *Phytomonas tumefaciens* on the beet root as a whole.

Table V gives the ratio of substance to crude protein (on weight basis). Since the crude protein can be considered as a measure of the living protoplasm in this material, these figures show the amount of substance present relative to the protoplasm.

TABLE IV

AMOUNT OF MATERIAL IN AN AVERAGE INDIVIDUAL UNDRIED BEET ROOT (EXPRESSED IN GRAMS)

| Constituent | Healthy beet root | Normal tissue of tumorous beet root | Tumorous tissue of beet root | Total in tumorous beet root |
|---|---|---|---|---|
| Average fresh weight | 17.97 | 14.59 | 11.48 | 26.07 |
| Average dry weight | 2.297 | 1.432 | 1.192 | 2.624 |
| Total ash | 0.222 | 0.193 | 0.157 | 0.350 |
| Free glucose | 0.023 | 0.012 | 0.014 | 0.026 |
| Free fructose | 0.013 | 0.015 | 0.009 | 0.024 |
| Sucrose | 1.101 | 0.425 | 0.147 | 0.572 |
| Starch | 0.027 | 0.034 | 0.029 | 0.063 |
| Ether extract | 0.036 | 0.027 | 0.031 | 0.058 |
| Crude protein | 0.239 | 0.173 | 0.239 | 0.412 |
| Pectins | 0.222 | 0.115 | 0.157 | 0.272 |
| Total uronic anhydrides | 0.288 | 0.163 | 0.154 | 0.317 |
| Uronic anhydride (in holocellulose) | 0.116 | 0.088 | 0.093 | 0.181 |
| Pentosans (in holocellulose) | 0.045 | 0.033 | 0.032 | 0.065 |
| Cellulose | 0.152 | 0.109 | 0.108 | 0.217 |
| Acid lignin | 0.093 | 0.070 | 0.071 | 0.141 |
| Total methoxyl | 0.020 | 0.013 | 0.015 | 0.028 |
| Methoxyl insoluble in anhydrous ether | 0.019 | 0.013 | 0.015 | 0.028 |
| Methoxyl insoluble in anhydrous ether and ammonium oxalate (0.5%) | 0.016 | 0.012 | 0.013 | 0.025 |

The average infected beet is heavier than the non-infected beet and contains a greater total amount of solid matter (Table IV). This agrees with the work of Levine (11). However the average infected beet contains only about one-half as much sucrose as the average healthy beet, although it contains more of all the other substances. This indicates that the crown gall organism stimulates the beet root to produce these substances from sucrose. Both protoplasmic and cell wall materials are formed.

The concentration of the ash elements is greatest in the tumorous tissue (Table III). Possibly it is because these are required to neutralize the acids which Brown and Quirk (4) have shown to be present in higher concentrations in tumours. The concentration of sucrose is lowest in tumours, being only one-fifth the concentration in the healthy living tissue of non-infected beets, and less than one-half the concentration present in the normal tissue of tumorous beets (Table III). It is interesting to note that the concentrations of glucose and fructose show no marked variation and that starch is actually present in increased concentration in the tumours (Table III). A noteworthy fact (Table III) is that the *normal tissue of tumorous beets* has a lower concentration of protoplasmic (crude protein, ether extract) and cell wall substances (lignin, methoxyl, cellulose, uronic anhydride, pentosans, and pectin) than either tumorous tissue or healthy tissue of non-infected beets. It also contains the lowest concentration of solids (Table I). From these results it would seem that the tumorous tissue robs the normal tissue of the same root of its stored sucrose and converts this into protoplasmic and cell

wall constituents in the tumour. Apparently the tumour does not stimulate the normal tissue of the same beet root to convert sucrose into these substances itself, at least not to any marked extent.

TABLE V

COMPOSITION OF TUMOURS AND NORMAL TISSUE OF BEETS EXPRESSED IN TERMS OF THE CRUDE PROTEIN

| Constituent | Healthy beets* | Normal tissue of tumorous beets* | Tumorous tissue* |
|---|---|---|---|
| Total ash | 0.93 | 1.11 | 0.61 |
| Free glucose | 0.10 | 0.07 | 0.06 |
| Free fructose | 0.05 | 0.08 | 0.03 |
| Sucrose | 4.51 | 2.45 | 0.61 |
| Starch | 0.11 | 0.20 | 0.13 |
| Ether extract | 0.15 | 0.16 | 0.15 |
| Pectins | 0.93 | 0.66 | 0.66 |
| Total uronic anhydride | 1.20 | 0.93 | 0.64 |
| Uronic anhydride (in holocellulose) | 0.48 | 0.50 | 0.39 |
| Pentosans (in holocellulose) | 0.19 | 0.19 | 0.13 |
| Cellulose | 0.64 | 0.63 | 0.45 |
| Acid lignin | 0.38 | 0.40 | 0.30 |
| Total methoxyl | 0.078 | 0.076 | 0.063 |
| Methoxyl insoluble in anhydrous ether | 0.078 | 0.075 | 0.063 |
| Methoxyl insoluble in anhydrous ether and hot ammonium oxalate (0.5%) | 0.068 | 0.070 | 0.053 |

*These figures are the ratio: $\frac{\%\ substance\ (dry\ weight)}{\%\ crude\ protein\ (dry\ weight)}$

The results of Table V show that the ratio of cell wall materials (cellulose, lignin, methoxyl, pentosans, and uronic anhydride (in holocellulose) ) to protoplasm is the same in the normal tissue of infected beets and the tissue of healthy beets but in the tumours there is a relatively greater concentration of protoplasm. This indicates the greater physiological activity to be expected in tumour cells and substantiates the well known concept that tumour cells are comparatively "young" cells. There is less than one-seventh the amount of sucrose per unit of protoplasm in tumours as in the healthy tissue of non-infected beets; the reducing sugars are also present in a lower amount per unit of protoplasm.

From Table II the ratio of cellulose to the other cell wall components can be calculated, and it will be observed that this does not vary much in the different tissues; this proves that no increase in cell wall incrustation takes place in tumour formation. The pectins show a peculiar behaviour. The ratios of cellulose to pectin in healthy beet tissue, normal tissue of infected beets, and tumorous tissue are respectively 0.68, 0.95, 0.68. Examination of the results in Table III indicates that pectins are apparently present in comparatively small amounts in the normal tissue of infected beets, thus indicating that the tumours have removed pectin from the normal tissue.

The values for total uronic anhydride reflect this change in pectin content. It is possible that it is necessary to distinguish between two different kinds of pectin:

*(a)* a reserve pectin,

*(b)* a structural pectin,

and to postulate the reserve pectin as that being transported to the tumours for transformation into other materials.

The results reported in this paper are in general agreement with those obtained by previous workers on this subject and the following observations seem warranted:

The crown gall organism exerts a local stimulus on the cells of the beet root causing them to proliferate, thus producing new cell wall and protoplasmic material. The latter is produced from sucrose supplied from the rest of the root; possibly pectins also are obtained similarly. The normal tissue of the tumorous root attempts to function normally and build up a store of sucrose. However considerable sucrose is lost to the tumour with the result that the plant as a whole probably manufactures much more sucrose than a non-infected plant since more dry matter is formed but, in the end, the infected plant contains less sucrose because it has been converted into other substances (protein, lipids, cell wall materials). This conversion of sucrose to proto plasmic and cell wall materials occurs in the tumorous tissue of the infected beet in which the normal tissue acts as a food reservoir. The normal tissue is apparently not stimulated by the bacteria to convert sucrose into other substances. Thus in the same beet root we have a tissue which is actively synthesizing new cell wall and protoplasm and a tissue of the same type that is relatively inactive, in this respect, and serves to store food.

Valuable information as to the nature of the mechanism of the conversion of sucrose into new living material might be obtained from a comparative study of the carbohydrate metabolism (respiration) of these tissues. It is quite possible that sugars are respired to give different intermediates in tissues that are synthesizing new material actively as compared with the intermediates formed in tissues whose chief function is to store sucrose, and beets infected with crown gall should provide suitable material for such a comparative study.*

## Tentative Conclusions

Presentation of results of the type obtained in this investigation based exclusively on percentage of dry matter does not show the true effect of tumour formation on the physiological activity of beet root. It is necessary to calculate the results in such a way as to permit comparison of concentration of materials in the living tissue, the amount in an individual beet root, and the ratio of one substance to another, before any valid conclusions can be drawn regarding the effect of *P. tumefaciens*.

The crown gall organism stimulates growth of the root as a whole; more solid matter is produced in infected roots.

* *This is now in progress.*

The tumour contains cells that actively convert sucrose into new protoplasm and cell wall material. The cells of the tumour are richer in protoplasm than those of mature normal tissue.

The crown gall organism stimulates the tumour cells only, causing them to proliferate and synthesize living material from stored food.

The normal tissue of tumorous roots loses sucrose and possibly pectins to the tumours but apparently is not stimulated by the bacteria to synthesize protoplasmic and cell wall material. The increased dry weight of tumorous roots is due to synthesis that occurs in the tumours. The rest of the root is stunted. There is no increase in the degree of cell wall incrustation in tumours. The cell wall of the cells in tumours and normal tissue is similar in chemical composition. There is a greater concentration of cell wall material and protoplasm in tumours and a smaller concentration of sucrose.

An infected beet root contains less sucrose (although more solid matter) than a healthy beet root.

One of the major metabolic processes in tumours is the conversion of sucrose into protein.

The lignin in beets is probably of the same general nature as the lignin in woods, as shown in the ethanolysis results in the dried beets.

It is the authors' intention to carry out a study of the carbohydrate metabolism of these tumours to determine the manner in which it differs from that of normal tissue. It is possible that such a study may serve to indicate the relation between respiration and synthesis.

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# Canadian Journal of Research

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## PARASITES OF SOME CANADIAN SEA MAMMALS[1]


BY L. L. LYSTER[2]



### Abstract

Four nematodes, two acanthocephalids and specimens of the cestode genus *Diphyllobothrium* are reported from seals and white whales in Arctic and temperate coastal waters of Canada. *Phocascaris netsiki* sp. nov. is described from *Phoca hispida* and observations on flagella in *Anisakis simplex* are recorded.


To the greater number of Canada's Arctic and sub-arctic inhabitants the sea-mammals supply staples both of food and clothing. In the course of our survey of parasites a considerable amount of material has been collected post-mortem, from these animals. Seals are represented by collections from several of the eastern Arctic posts. This material (as was the single parasite-free walrus examined) was secured chiefly with the co-operation of the Royal Canadian Mounted Police and the Hudson's Bay Company who sent formalized viscera to the Institute. In addition, collections were made by Dr. Parnell in 1934 (10) and by the author in 1939. White whale parasites, collected in the Gulf of St. Lawrence, were supplied to us by Dr. V. D. Vladykov and viscera of whales have also been received from the eastern Arctic posts.

Formalization of viscera leaves much to be desired from the standpoint of the condition of the material collected, but it has made available resources and collecting points that would not otherwise have been reached. The sources of the present material, as listed with the hosts, have been mapped in an earlier paper (4).

Parasites recovered during the survey included nematodes, cestodes and acanthocephalids. Trematodes are conspicuously absent; our correspondence files include reference to "liver flukes" of seals at Cape Smith, but our post-mortem studies on livers from this area, though showing fatty degeneration and necrosis, have produced no trematodes. Personal notes also include a report by a Cape Smith native who described white rough livers in local seals, a condition which, according to the Esquimaux, renders the entire carcass unfit for food.

In fish-eating seals and white whales, nematodes invariably occurred in the stomach in close association with the partially disintegrated portions of fish


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[2] *Graduate assistant. (Now deceased.)*

found there. In many cases these lodged so firmly in the fish muscles that it was necessary to dissect them out. These nematodes are adult forms and therefore not introduced with the food. Whether they feed on the fish ingested by their host, act as symbionts serving to break up the bolus, or whether there is some more obscure relationship, remain interesting speculations. Extracorporal digestion among ascarids has been noted by Hoeppli (7) among *Contracaecum* sp. in seal stomachs. The nematodes he studied were actively parasitic on the host, but the fact is established that they are capable of producing histolytic changes in an external medium. The nematodes concerned must be highly anti-proteolytic; apparently healthy individuals are to be found in the oesophagus, stomach, and small intestine of the more heavily infected animals.

## Nematodes

Three species of different genera are included in our seal collections and one species is reported from white whales.

*Porrocaecum* Railliet & Henry, 1912.

The most common parasite of the bearded seal belongs to the genus *Porrocaecum* on the criteria of the presence of a single intestinal caecum and dentigerate lips. The single species of the genus reported from Arctic seals has been discussed by Baylis (1) who reviewed the earlier studies and material and differentiated the species largely on the structure of the lips and alimentary tract. Since establishment of the genus, many major points of species differentiation have become generic and it is difficult to refer the present material to the species *P. decipiens* described by Baylis; a detailed description is accordingly given.

The male is 9 to 11 mm. long by 0.5 to 0.6 mm. wide in greatest measurement. The female is about 15 mm. long by 1 mm. wide, with a maximum specimen of 37 to 1.25 mm.

The two subventral lips are triangular in shape, not unlike a conventional heart, apex toward the base, anterior margin cut medially to make two prominent lobes. The pulp of the lip follows the same outline, leaving a semitransparent margin. A prominent single papilla is situated at the apex of the triangle. The anterior margin of the dorsal lip is similarly cleft, but the base of the lip is wider than the free portion. About midway the length of the dorsal lip there is an external thickening behind which two papillae are situated. No interlabia are present. Dentigerous ridges vary in arrangement and extent. In some specimens they are to be seen only at the contact of the anterior lobes; in others they are continuous throughout the anterior margins while in one case they outline the lateral margins of each lip. In some specimens it was impossible to find any evidence of dentigerous ridges, though other features were constant. These structures were never very conspicuous.

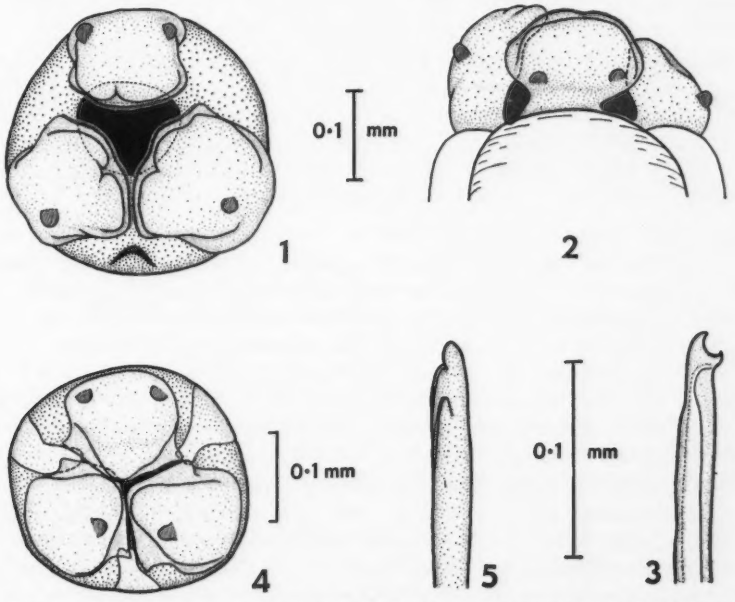
The oesophagus is divided into the usual two structurally different parts, continuous with each other, and opening without change of direction into the

intestine. The anterior portion is 1.7 mm., the posterior 0.15 mm., in length. At the point of origin of the intestine rises a diverticulum which runs anteriorly to a point approximately in line with the limit of the glandular oesophagus, a distance equal to about one-fortieth the body length.

The female tail is bluntly rounded with a small terminal knob. The vulva is one-third, or somewhat less, the body length from the anterior end. The genital structures are as figured by Baylis.

The male body terminates in a sharp point but is given a blunt appearance by a cuticular wing, 0.03 mm. at its widest point, which is continuous around the end of the body, from a point roughly in line with the cloacal opening. The tail is 2.5 mm. long carrying seven papillae; three are lateral and paired, one is medial and almost terminal. More or less parallel with the spicules four pairs of papillae are situated on either side of the cloaca and a single large one is central and just anterior to this opening. The greatest number of pre-anal papillae found in any of our specimens was 52 pairs. For some distance anterior to the cloaca they occur in a regular line parallel to the sides of the body, but about the thirtieth they tend to become scattered into a general plan of two rows in which the papillae alternate in pairs.

The spicules are equal, 1.5 mm. long. They are straight to the tip, with lateral alae which curve ventrally, meeting just before the end. Terminally




FIGS. 1–3. *Porrocaecum decipiens ex E. barbatus. 1. En face view. 2. Head, dorsal view. 3. Spicule.* FIGS. 4–5. *Contracaecum osculatum ex P. vitulina. 4. Head, en face view. 5. Left spicule.*

the spicule broadens and a semilunar indentation gives it a clawed appearance.

These specimens are notably smaller than those discussed by Baylis. This is a fact for which it is difficult to find an explanation; even although no well-developed eggs were noted, it seems unlikely that the increase in size to complete maturity, would be as much as two to three times. Relative measurements, a more satisfactory criterion, correspond with those given by him.

The main lips are not identical with those discussed by Baylis and there is variation in the numbers of pre-anal papillae; these two factors are considerably influenced by fixation within the limits of the present differences.

Because of general head structure, papillary arrangement and, notably, on biological grounds, the present material has been considered to be the species *P. decipiens*. The variations noted are not of sufficient significance to allow specific differentiation and Arctic seals are considered as carrying the single species.

Less mature specimens of the same species were recovered from the stomach of most of the individual animals studied. These were usually attached to the mucosa in bundles of about 20 worms. The largest specimen occurring in this site measured 1.8 by 0.08 mm.; the oesophagus was 0.28 mm. and the caecum 0.04 mm. in length. The larger number of these forms was about half this size. Specimens found unattached in the stomach included many enclosed in their larval sheath, and adults were also recovered here. A single specimen was taken from the oesophagus of a Jar Seal *(Phoca vitulina)*.

Most specimens of *Erignathus barbatus* that we have examined are parasitized by this nematode; these have come from ports on either side of Hudson Strait. In addition, we have records of it from *Phoca groenlandica*, Stupart Bay, from "Seals", Havre St. Pierre, Que., and Craig Harbour, and from a "Jar Seal" taken at Wolstenholme. Because of feeding habits, etc. of *P. vitulina* the "Jar Seal" would seem to be a mistaken identification of the host.

*Contracaecum* Railliet & Henry, 1912.

This genus has been fully discussed by Baylis (3), who lists three species from pinniped hosts. The present material corresponds most closely to his description of the northern species *C. osculatum*. The dorsal lip is of relatively uniform width with sharp cuticular lobes, the oesophagus is cylindrical, the spicules over nine mm. long; these are features on which he bases the identification. In over-all measurements and in most details of structure the present specimens correspond exactly with that species. Some differences in detail, however, must be noted.

In the male specimens, averaging 40 mm. long by 1.8 mm. wide, in no instance were there as many as 50 pre-anal papillae; they numbered about 40. Wrinkling and shrinking of the cuticle obscures these structures and will account for at least some of the difference. The spicules were slightly longer than Baylis records, ranging up to 10.3 mm. The spicular alae do not reach the end of the spicule and that on the right does not extend so far as that on the left.

In the female, up to 60 mm. long by 2.5 mm. wide, the vulva is situated at about the limit of the anterior sixth of the body. The oviduct divides, each portion entering a branch of the uterus, at about one-third the body length. Near this point the ovaries rise and proceed backwards to within 9 mm. of the posterior end (in a 60 mm. specimen), then run forward about 5 mm. to meet the uterine tubes.

In spite of the variations (which are readily attributable to different methods of fixation) since over-all characteristics and particularly the structure of the lips, are so closely identifiable with Baylis's detail for *C. osculatum*, it is to this species that the present material has been assigned.

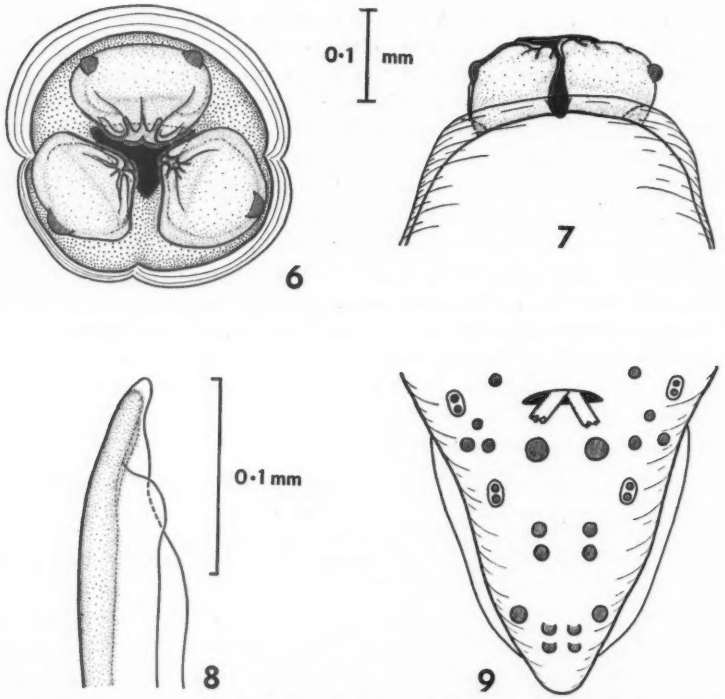
The species has been recorded in the survey from *P. hispida* and *P. vitulina*. It is found from Dundas Harbour, Devon Island, and Craig Harbour in the north to Havre St. Pierre, Que., in the south. Intermediate points of collection have been Lake Harbour, Clyde River in Baffin Island, and Stupart Bay and Wolstenholme on Hudson Strait.

*Phocascaris* Höst, 1932

This genus has hitherto included the single species *P. phocae* reported by Höst from *P. groenlandica* (8). It is very like *Contracaecum* in conformation of alimentary tract but because of modified and dentigerate lips it cannot be placed in that genus. *Phocascaris*, accordingly occupies a valid place among the Anasikinae.

Several specimens collected from seals in the Canadian north show the features that characterize this genus. None of them, however, conform exactly to Höst's description of the species (8). In *P. phocae* the anterior margins of the lips are formed by four labial folds decorated with dentigerous ridges and each subventral lip carries a large and a small papilla. In the present material the anterior margins of the lips are entire with an occasional exception in the case of the dorsal lip when a medial indentation may occur. Dentigerous ridges are continuous from about the midlateral areas of each lip. Somewhat removed from the margin, six labial folds are conspicuous, the outermost pair being closely applied and outlined by dentigerous structures giving the effect of four submarginal scallopings. A single large papilla is to be seen on each subventral lip and a pair of double papilla lateral to the male cloaca are further features that distinguish this material from *P. phocae*, in which no comparable structures were seen. These differences and the size are sufficient to make it impossible to identify these specimens as belonging to Höst's species, and it is accordingly necessary to create a new species to designate our collection. For this the author proposes the name *P. netsiki* sp. nov.

The female is 30–76 mm. long with a maximum width of 0.6 to 1.2 mm.; male specimens measure 20 to 51 mm. long. The oesophagus, about one-tenth to one-fifteenth the body length, often coils upon itself midway in its course. The proventriculus about one-eighth the length of the oesophagus proper, opens without change of direction into the intestine. An intestinal




FIGS. 6 – 9. *Phocascaris netsiki ex P. hispida. 6. En face view. 7. Head, ventral view. 8. Spicule. 9. End of male, showing peri-cloacal and terminal papillae.*

caecum is equal to or only slightly greater than the proventriculus. A wide oesophageal diverticulum runs posteriorly a distance equal to half the length of the oesophagus proper, terminating in a sharply recurved distal point. Inconspicuous lateral cephalic alae occur.

The female genital complex varies in organization from that described for *P. phocae*. The oviduct opens by a narrow constriction into a Y-shaped uterus. The two posterior branches of the uterus are joined by the much smaller ovaries which enter dorsally and just short of the ends of the branches. The ovarian tubes follow a convoluted course posteriorly, almost to the anus, then run directly forward to the level of the oviduct. The vulva is situated about one-fifth the body length from the anterior end. The eggs are spherical or slightly ovoid, thin-shelled, containing a segmented ovum; they are about 0.08 mm. in diameter.

The male spicules are long, sub-equal and simple. They are alike in structure. A strongly chitinized dorsal rod is continuous ventrally with a semitransparent hollow tube. This latter divides about 1 mm. from the end to form two lateral alae. One of these is extended around the end, the

other is discontinued just anterior to the end of the spicule. In a specimen 51 mm. long the left member was 5.0 mm. and the right 5.5 mm. long. They average about one-tenth the body length. Five pairs of single and one pair of double papillae decorate the tail and a similar number are peri-cloacal. of the last a pair of double papillae are laterally in line with the cloaca. Fifty pairs of single pre-cloacal papillae follow a regular course parallel to the sides of the body. Narrow lateral caudal alae are present.

In both sexes the cuticle is finely striated throughout; these striae coupled with close cuticular folds, form a distinct collar around the base of the lips.

Infections with *P. netsiki* were never heavier than five to ten worms in a host. They were found in *Phoca hispida* from Craig Harbour, Clyde River, and Lake Harbour, N.W.T., and in a "seal" from Cape Smith, N.W.T.

*Anisakis* Dujardin, 1845

Several thousand ascarids collected from the alimentary tract of *Deliphinapterus leucas* are identifiable as belonging to the genus *Anisakis*, on a basis of absence of alimentary diverticula and interlabia and presence of dentigerous ridges and a two-part oesophagus.

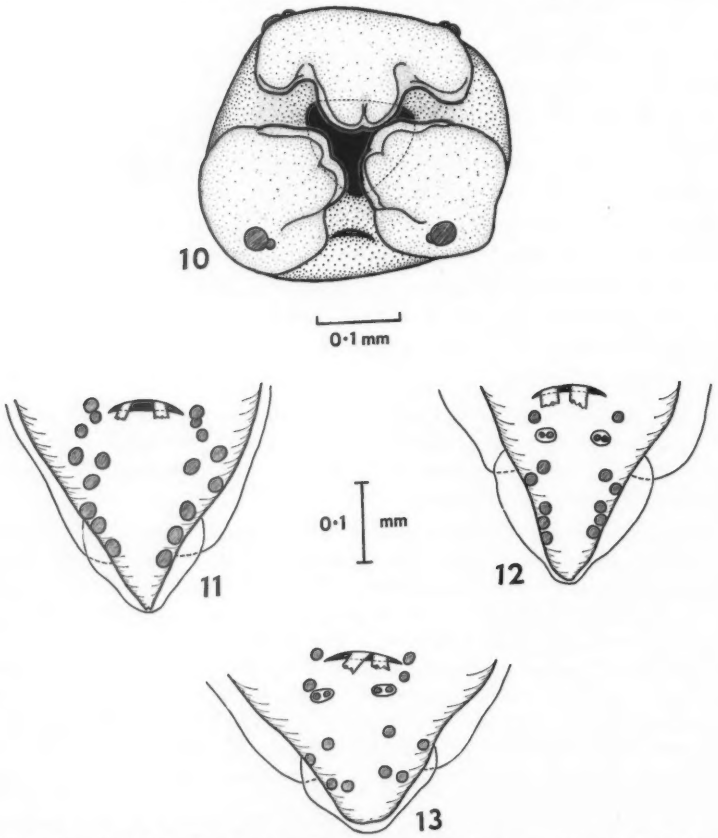
From marine mammals eight species of this genus have been described, but marked confusion of early descriptions and later synonymities make specific determination almost impossible. Three species, *A. similis*, *A. rosmari* and *A. physeteris* have been reported from carnivorous mammals, and one, *A. insignis*, from a freshwater dolphin. The remaining four species have been described from various porpoises and small whales, and it is among these that most confusion has arisen. *A. dussumierii* Van Beneden, 1870, was first described as *A. simplex* by Dujardin; *A. typica* Diesing, 1860, has been listed as a synonym of *A. dussumierii* by Stiles and Hassall (11) and *A. kukenthalii* Cobb, 1888, has been proposed as a synonym of *A. simplex* Rudolphi, 1809, by the same authors.

These four species are not only poorly differentiated, but they have a great deal in common. The problem of assigning the present material to a place in the genus is one of selecting constantly differential characteristics and interpreting them to apply to our specimens. Table I, correlating the measure-

TABLE I

COMPARATIVE MEASUREMENTS OF *Anisakis* FROM MARINE CETACEA (MM.)

| Male | Female | Oesophagus I | Oesophagus II | Spicules | No. post. papillae | No. prepapillae | Width striae | Species |
|---|---|---|---|---|---|---|---|---|
| 31–70 | 37–90 | 4 | 1.25 | 3.9 and 0.96 | 6–10 | 75 plus | 32μ | *typica* |
| 79 | 70–100 | 5.5 | 1.5 | 2.7 and 1.5 | 8–10 | ? | 30μ | *dussumierii* |
| 37–130 | 79–200 | | | 1.68 | 6–8 | 50 plus | 23μ | *simplex* |
| 70–90 | 80–100 | 5.1 | 2.5 | 2.3 and 1.7 | 7–8 | 90 | 30μ | *kukenthalii* |
| 60–90 | 63–110 | 5.4 | 1.6 | 2.0 and 1.4 | 7–9 | 56–90 | 23μ to 38μ | Present form |




FIGS. 10 – 13. *Anisakis simplex ex D. leucas. 10. En face view. 11 – 13. End of male showing progressive modifications in papillary arrangement.*

ments given for cetacean-parasitizing *Anisakis* in the literature (notably, in Stiles and Hassall (11) ), illustrates the inadequacy of measurement in specific diagnoses. (A similar table is used by Baylis (2) in establishing his species *A. physeteris*). The minimal measurements of 31–79 mm. in the male and 37–80 mm. in the female and the maximal measurements of 70 to 130 mm. and 90 to 200 mm. in the female will not exclude a single species. Similarly measurements of structures and papillae counts are non-differential. If any species is in any way distinct it is *A. typica*, on a basis of insignificantly smaller size, and its separate identity from *A. dussumierii* has already been questioned. In the same way, arrangements of structures do not offer a means of separation. No detail of labial structure has been held significant except the tendency for greater distinction between the basal and lobate portions of the dorsal lip illustrated by Krabbe (9) and by Stiles and Hassall working with "*A. typica*".

The female worms of the group are undifferentiable from descriptions. The variations in spicule length and papillae arrangement in the males are limited to differences within definable limits into which all species will fall.

Because they are found in a closely related group of circumpolar animals of similar habit, and because there seem to be no satisfactory morphological criteria for recognizing distinct species, it seems unlikely that these four members of the genus are dissimilar beyond the insignificant variations that are shown within most nematode species. This view is further borne out by the considerable differences that have been noted between these four members of the genus on the one hand and *A. similis*, *A. physeteris*, and *A. rosmari* on the other, a differentiation that is to be anticipated on biological and ecological, quite apart from morphological grounds.

For these reasons the present material has been assigned to the species *A. simplex* which is here considered to have as synonyms *A. kukenthalli*, *A. typica*, and *A. dussumierii*.

Males in our collection measure 60 to 90 mm. by 1.7 to 2.4 mm., females 63 to 100 mm. by 2.0 to 2.6 mm. The oesophagus has an anterior muscular portion averaging about 5.5 mm. long, and a posterior, usually laterally sigmoid, proventricular portion 1.5 mm. long, or slightly greater. Cuticular striae 23 to 38$\mu$ apart are continuous throughout the body length. The three lips follow the usual typical pattern; a crescentic dorsal lip 0.24 by 0.12 mm. bears a pair of anterior projections medially; two subventral lips are more nearly round in outline, with a pair of lobes reduced in extent but similar to those of the dorsal lip. Dentigerous ridges outline the opposed margins of these lips. The dorsal member carries a pair of papillae at the lateral corners and each subventral lip is decorated with a pair of ventral papillae. A pair of cervical papillae is about 1.0 to 1.5 mm. removed from the base of the lips.

The vulva of the female opens just in front of the middle of the body (45 mm. from anterior end in a specimen 105 mm. long). An oviduct about 12 mm. long loops longitudinally before entering the bicornate, posteriorly directed uterus about 33 mm. long. Each uterine branch is joined by a long, slender and highly convoluted ovary which runs backward for a short distance, then to the forepart of the body well anterior of the vulva. Intrauterine eggs are subspherical, 0.045 to 0.5 mm. in diameter.

The male spicules are slightly curved, slender, dissimilar; the left is 2.0 mm. or slightly more, the right 1.2 to 1.4 mm. in length. Sixty to ninety pairs of pre-anal papillae have been counted, the greater number of specimens having a larger number of these structures.

The tail of the male is relatively short, 0.18 to 0.22 mm. long. A narrow ala, continuous around the end of the tail is about 0.04 mm. in marginal extent. It meets lateral alae 0.02 to 0.03 mm. wide that terminate just anterior to the cloaca. Pre-cloacal papillae number 60 to 90 pairs reaching an anterior limit 5.0 mm. from the end of the body. Post-cloacal papillae

numbering six to eight pairs, differ in arrangement. Nearest the tip in some specimens three pairs occur closely in line parallel to the body margin; a fourth marginal pair is anterior to these and a fifth pair lies anteriorly toward the midline. Two pairs extend from below the lateral margins of the cloaca toward the alae and just at the corners of the cloaca two pairs are in close contact. The third of the three posterior pairs is not infrequently absent; in specimens showing this condition the papillae are arranged in well defined groups of two, of which the immediately post-colacal group sometimes includes a pair of double papillae toward the midline. No other differences may be noted in association with these variations in papillary arrangement.

In these helminths the male genital tract shows a noteworthy condition. The vas deferens, immediately after its origin, is folded in a longitudinal sigmoid curve from which it proceeds directly to the cloaca. Within a limited area at the beginning of the direct portion of the deferens the lining epithelium is decorated with hair-like processes. These occur in clusters of about 30 placed about 25$\mu$ apart in longitudinal rows. The rows, 35$\mu$ apart, are laterally staggered, so that the groups of processes alternate around the organ. The individual processes are relatively uniform in length, from 60 to 65$\mu$; 25 groups of these structures may be seen in cross section.

The material now available has all been preserved in formalin for a considerable time and it is not possible to determine whether these structures are, as their appearance strongly suggests, flagella. In describing "*Ascaris kukenthalii*" Cobb (5) noticed similar forms. His descriptions and illustrations prove that the structures he saw were identical with those in the present material. Apparently using living material, he reported motility for the processes and even attributed a definite function to them, that of assisting the movement of the spermatozoa on their way down the vas deferens. He says in part: "This" (the epithelium of the posterior part of the vas deferens) . . . "is provided with bundles of strong cilia which remind one of the not dissimilar elevations of the epithelium in *Ascaris lumbricoides*. In that case, however, the elevations are branching and show, according to Schneider and Lueckart, only amoeboid movement. Here, on the other hand, only bundles of often true cilia occur to which I must attribute a very active movement, not only because they show the composition of real cilia, but also, mainly because the free ends are directed posteriorly, and we find all the spermatozoa assembled in a central longitudinal bundle. In the space between the axial mass of sperms and the wall of the duct, i.e., in the space in which the cilia move, there are no spermatozoa."

Then follows a discussion of the grouping and extent of these structures, parallel in every way with those the author has noted. The observations of the author are further comparable in that the spermatozoa are invariably swept to the centre of the duct as Cobb has described.

Hetherington (6) in a discussion of ciliation quotes Cobb in an 1898 paper in which he discusses the motility of structures in the seminal vesicle without apparent reference to the worm in which he noted the condition; it would

seem that Cobb had in mind the observations quoted here and of which Hetherington was apparently unaware. The length of these processes, as well as their structural arrangement in distinct tufts, readily suggests their classification as flagella purely on the basis of appearance and arrangement. If, then, the evidence of Cobb's paper for their motility is accepted it must be concluded that this nematode species, at least, has flagella.

By far the greater bulk of our *D. leucas* material originated in the Gulf of St. Lawrence. Viscera of only three white whales have been available from other sources. Of these, one from Southampton Island, N.W.T., was parasite-free, one from Craig Harbour, Ellesmere Island, furnished a single immature *Anisakis* and the third, with origin at Lake Harbour, Baffin Island, was parasitized by a few immature *Anisakis* sp.

## Acanthocephalids

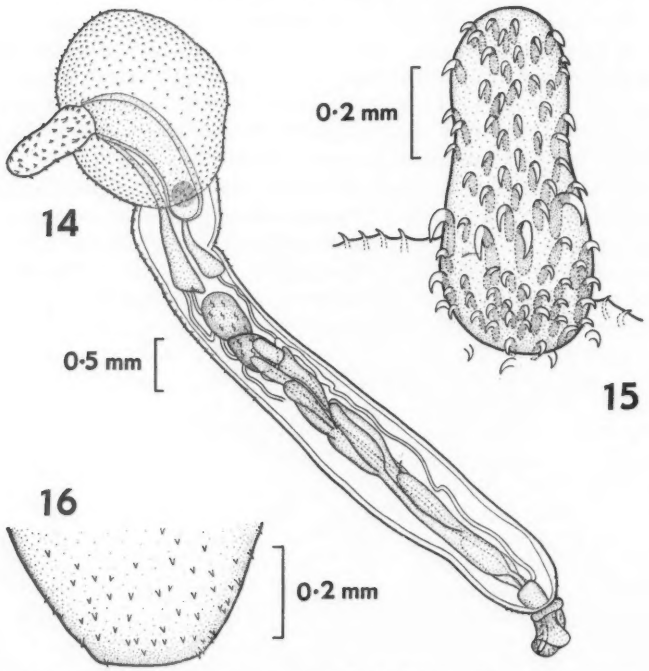
*Corynosoma* Luhe, 1911

Fixed firmly in the mucosa of the small intestine of seals, acanthocephalids of the species *C. strumosum* were collected from *Erignathus barbatus* taken at Cape Smith, Craig Harbour, Clyde River, and Pond Inlet, and from *Phoca hispida* at Cape Smith, Lake Harbour, Payne Bay, Stupart Bay, and Wolstenholme. *C. semerme* was found only once in a specimen of *E. barbatus* taken at Lake Harbour in August, 1939. In an earlier paper (4) an infection of *C. semerme* from dogs at Craig Harbour is recorded; though some of the seal acanthocephalid material from this post is very badly preserved there is little doubt that the specimens are *C. strumosum* rather than *C. semerme.* Though none of the Arctic material from *Delphinapterus leucus* included acanthocephalids, white whales in the Gulf of St. Lawrence were commonly parasitized by *C. strumosum.*

*Corynosoma strumosum* Rudolphi, 1892

Males are about 7 mm. long by 1.2 mm. wide in the region of the testes; females slightly larger when gravid. The body is straight, with almost parallel sides, up to an expansion at the anterior end where the body width is doubled. The proboscis is slightly barrel-shaped 0.85 mm. long by 0.35 mm. at its widest points. Spines in 18 rows decorate the organ; these are smallest at the base of the proboscis, extending about 0.04 mm. from the cuticle; at the fourth row they increase in size to 0.09 mm. then gradually decrease in size until, at the anterior tip, they are about 0.05 mm. The root of these spines is always about one-fourth greater than the free portion.

The proboscis narrows to the anterior third, then increases in size toward the base. The anterior expanded portion of the worm is thickly armed with small spines set deep in the cuticle; these increase in size at the base of the proboscis, and are continued into the body of the worm to a maximum point just below the posterior testis in the male and a corresponding position in the female. Spination is renewed terminally in the male. The proboscis sheath is relatively short, reaching a point just behind the testes; the lemnisci are truncated, not reaching the testes.



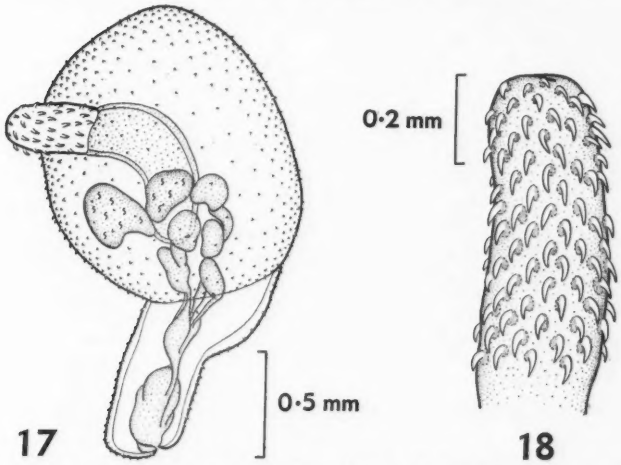

FIGS. 14 – 16. *Corynosoma strumosum ex E. barbatus.* 14. *Entire worm, male.* *15. Proboscis.* *16. Detail of terminal spination, male.*

The two testes of the male are roughly rectangular in shape, 0.5 by 0.3 mm., longitudinally colinear and in close contact with each other. The numerous cement glands form a compact mass running from the level of the middle of the posterior testes to the posterior end. Retinaculae follow an irregular course posteriorly from the brain. A conspicuous copulatory bursa is present. The female, apart from sexual structures, including the absence of terminal spines, is not dissimilar from the male. The body contains many embryos about 1.08 mm. by 0.5 mm. in size.

*Corynosoma semerme* Forssell, 1904

Males measure about 1.3 to 2.0 mm. by 0.9 to 1.0 mm.; females 2.8 to 3.0 mm. by 2.0 to 2.2 mm. The present specimens have been found to compare with the *C. semerme* material from dogs identified by Dr. Van Cleave. They have been assigned to this species on the criteria of size and shape and the specific arrangement of spines on the proboscis and body.

The short body, made up largely of an anterior expansion with a truncated rear-body is roughly comma-shaped. Spines in both sexes are continued into the rear quarter of the body dorsally and terminally on the lower side; in the male and female the end of the body is spined. The proboscis is armed with




FIGS. 17 – 18. *Corynosoma semerme ex. E. barbatus.* 17. *Entire worm, male.* 18. *Detail of proboscis.*

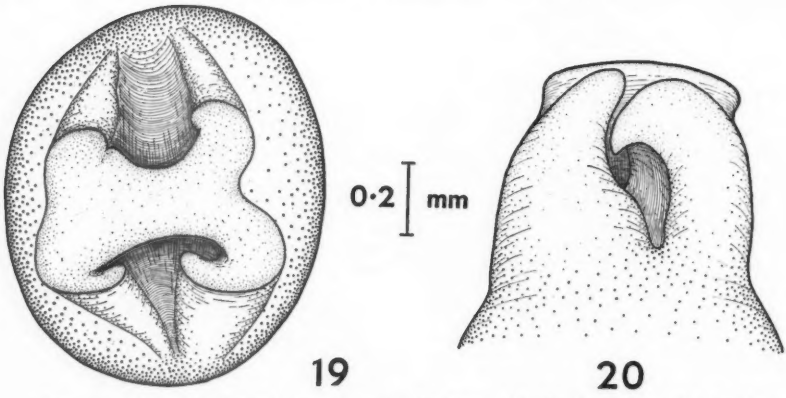
19 rows of spines. These are smallest in the first two rows (0.025 mm.), the remainder being uniform in size (0.062 mm.). The roots of these spines are never greater than about half the length of the free portion. The proboscis itself is distinctly more uniform in diameter than is the case with *C. strumosum* and measures about 0.7 by 0.25 mm. Organs are essentially similar to those of *C. strumosum* but more compactly arranged. The testes are laterally colinear, the lemnisci and proboscis sheath lie dorsally, and extend somewhat posterior, to them.

## Cestodes

Cestodes require more careful technique in preserving and fixing than collections of this kind provide. For this reason, although considerable material has come to hand, all of it is in too poor condition to permit with certainty diagnosis though it is identifiable beyond doubt as falling within the genus *Diphyllobothrium*. All specimens are much contracted and in most decomposition has occurred to a lesser or greater extent. The collection is made up largely of broken strobila, some with the scolices included. Several specimens are complete animals; these are made up of more than 75 segments and in their contracted condition measure about 25 by 6.0 mm. The eggs in balsam mounts measure about 0.053 by 0.035 mm.; there was no significant variation from this size.

In most cases three to four uterine loops may be noted but occasionally there are as many as seven. In all cases the uterus lies definitely behind the cirrus sac, the uterine pore lying ventral to about the left rear margin of the sac.

The bothria are deep, separated by a frontal ridge, but reaching the anterior limit of the scolex in lateral perspective and may be traced to the posterior




FIGS. 19 – 20. *Diphyllobothrium sp. ex E. barbatus.* 19. *En face view.* 20. *Scolex, ventral view.*

margin of the scolex. The scolex overlies the anterior part of the first segment behind. In heavily contracted specimens the scolex was roughly triangular in shape; isolated scolices were rectangular.

The differences in shape of the scolex and in the number of uterine coils would, by some standards, require species distinction. Stunkard and Schoenborn (12) have included these characteristics under *D. lanceolatum* and its synonyms, *D. schistochilos* and *D. coniceps*. Until it is possible to examine fresh material a diagnosis of this material as *D. lanceolatum* is indicated, and all distinguishable details conform.

The measurements do not disagree with the range listed for *D. lanceolatum*. The testes occupy a single medullary layer. The cirrus sac has a maximum diameter of 0.12 mm. with a relatively long, usually protruded cirrus; the double ovary has entire margins.

Further accurate details are not distinguishable.

The proglottids are longer than wide (1.8 by 2.4 mm. or wider than long (3.6 by 1.6 mm.). In a complete and highly compressed animal the proglottids were widest at about half the total length and were 0.4 mm. long and 5.5 mm. wide; the subterminal segment was 0.6 mm. long and 1.0 mm. wide.

Cestodes of this genus were recovered in *Erignathus barbatus* from Cape Smith, N.W.T., Cape Dorset, Baffin Island, and Wolstenholme, Que., and in *Phoca groenlandica* from Lake Harbour and Clyde River, Baffin Island.

The following host-parasite list summarizes our definite records in Canada to date:

*Delphinapterus leucas* (White Whale)
*Anisakis simplex*
*Anisakis* sp. inq.
*Corynosoma strumosum*

*Erignathus barbatus*
*Porrocaecum decipiens*
*Corynosoma semerme*
*C. strumosum*
*Diphyllobothrium* sp.
*Phoca vitulina* (Harbour or Jar Seal)
*Contracaecum osculatum*
*Phocascaris netsiki*
*Phoca groenlandica* (Harp or Greenland Seal)
*Porrocaecum decipiens*
*Diphyllobothrium* sp.
*Phoca hispida* (Ringed Seal)
*Contracaecum osculatum*
*Corynosoma strumosum*
*Phocascaris netsiki*

Of these, three records are new, the occurrence of *C. strumosum* in *D. leucas*, of *P. netsiki* in *P. hispida* and of *C. semerme* in *E. barbatus*.

## Acknowledgments

The active co-operation of the Commissioner and men of the Royal Canadian Mounted Police, of the Fur Trade Commissioner and traders of the Hudson's Bay Company and of Dr. Vladykov and his colleagues was invaluable in collecting the material upon which this paper is based. The author is also keenly appreciative of the privileges extended by the Administration of the Northwest Territories associated with his appointment to the 1939 Eastern Arctic Patrol, which allowed personal collecting in Ellesmere, Baffin, Somerset and Southampton Islands and the western coast of Hudson Bay from the R.M.S. "Nascopie".

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# THE DISTRIBUTION OF THE RED AND YELLOW COLOURS OF THE MUSCLE TISSUE OF BRITISH COLUMBIA CANNED SPRING SALMON AROUND THE MEANS OF THE INDIVIDUAL DISTRIBUTIONS OCCURRING IN SMALL, ARBITRARILY CHOSEN TIME INTERVALS[1]

By F. Charnley[2] and Laura M. Harcus[3]

## Abstract

The distributions of the red and yellow colours of the cooked muscle tissue of British Columbia spring salmon *(Oncorhynchus Tschawytscha)* around the means of the individual distributions occurring in small, arbitrarily chosen time intervals are composite distributions each consisting of two component normal distributions, thus indicating that there are two, and only two, varieties of this species when the salmon are classified on the basis of these two quality characteristics. The proportions of the pale and red varieties are very nearly 1 : 3 so that when sampling fluctuations are taken into account the data are in complete agreement with the hypothesis that the true proportions are, respectively, $\frac{1}{4}$ and $\frac{3}{4}$.

In an investigation of the functional forms of the distributions of the red and yellow colours of the muscle tissue of British Columbia canned salmon in connection with the grading of these characteristics, it was found that the distribution of the red colour of canned spring salmon *(Oncorhynchus Tschawytscha)* is markedly bimodal. The general shape of the distribution suggested that it was a composite distribution consisting of two component normal distributions with the red component forming considerably more than half the total population. If this hypothesis is correct and the species is homogeneous biologically, the data would indicate that the pale and red colours in this case are alternative Mendelian characters.

Before an answer to this last question can be undertaken, however, it is necessary to show that the distribution of the red colour in this species is in fact a mixture of two populations. Accordingly, the object of the investigation reported in this paper was to test the hypothesis as regards the functional form of the red colour and also to investigate that of the yellow colour of this species by fitting the experimental data with composite distributions made up of two component normal distributions.

## Selection and Tabulation of Data

The data employed in the investigation were taken from the records of routine examinations of British Columbia canned spring salmon for the 1938 season and consist of the colour measurements made during these

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*Contribution from the Canned Salmon Inspection Laboratory, Department of Fisheries, Vancouver, B.C.*

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examinations. The measurements were carried out by means of an Armstrong Colorimeter, an instrument (1) that utilizes the principle of matching the colour of the sample against coloured glasses. The intensity of colour is accordingly expressed in red and yellow Lovibond units.

In tabulating the original data preparatory to investigating the distributions of the red and yellow colours of the cooked muscle tissue of this species it was necessary to eliminate, as far as possible, three types of variations in the results. These were: (i) Variations arising from the fact that a number of companies pack only the red variety of this species, or, at least, only those salmon which, as far as can be determined, will definitely show red muscle tissue when cooked. (ii) Variations arising through geographical effects. For example, sockeye salmon caught in the northern areas in British Columbia are somewhat less intensely coloured than salmon caught in the southern areas, or in the Fraser River. The same seems to be true also of spring salmon. (iii) Variations resulting from seasonal effects.

Variations due to the first of these factors were eliminated by making careful inquiries of the companies who, as a result of preliminary inquiries, were believed to pack a certain proportion of, or all, unselected spring salmon. This information gave the canneries, in the area chosen, that had packed only unselected spring salmon during the 1938 season.

Variations resulting from the second factor were eliminated, as far as possible, by restricting the samples included in the investigation to those from the canneries in the area north of Knight Inlet that packed unselected samples during the 1938 season.

TABLE I

DISTRIBUTIONS OF THE RED COLOUR OF THE MUSCLE TISSUE IN UNSELECTED SAMPLES OF BRITISH COLUMBIA CANNED SPRING SALMON PACKED IN CERTAIN CANNERIES IN THE NORTHERN AREA DURING 1938

| Red colour, Lovibond units | Interval No. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| 1.5 | 1 | 1 | 2 | 2 | 1 | | | 1 | 2 | 1 | | | | | | | 2 |
| 2.0 | 5 | 1 | 1 | 7 | 5 | 4 | 2 | 1 | 2 | 3 | 3 | 3 | 4 | 4 | 1 | | 2 |
| 2.5 | | 2 | 2 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | 5 | 1 | 1 | 2 | 1 | | 4 |
| 3.0 | 2 | 1 | 2 | | | | 1 | 2 | | 4 | 5 | 1 | 3 | 2 | 1 | 4 | 3 |
| 3.5 | 4 | 1 | | | 2 | 1 | 2 | 1 | 1 | 2 | 3 | 2 | 4 | 1 | 2 | | 2 |
| 4.0 | 6 | 2 | 5 | 4 | 3 | 1 | | 4 | 2 | 2 | 6 | 3 | 1 | 2 | 2 | | 4 |
| 4.5 | 8 | 2 | 6 | 9 | 1 | 3 | 8 | 2 | 4 | 13 | 12 | 2 | 5 | 2 | | 2 | 2 |
| 5.0 | 4 | 4 | 6 | 2 | 4 | 3 | 7 | 6 | 6 | 5 | 10 | 5 | 4 | 3 | | | |
| 5.5 | 3 | | 1 | 5 | 1 | 3 | 1 | 2 | 2 | 10 | 1 | 2 | 2 | 1 | | | 3 |
| 6.0 | 1 | | 1 | 2 | | 1 | | 2 | 3 | 2 | | 1 | | | | | |
| 6.5 | | | | | | | | | | | 1 | | | 1 | | | 1 |
| 7.0 | | | | | | | | | | 1 | | | | | | | |
| 7.5 | | | | | | | | | | 1 | | | | | | | |
| Total | 34 | 14 | 26 | 32 | 19 | 17 | 22 | 22 | 24 | 46 | 46 | 20 | 24 | 18 | 7 | 6 | 23 |

TABLE II

DISTRIBUTIONS OF THE YELLOW COLOUR OF THE MUSCLE TISSUE IN UNSELECTED SAMPLES OF BRITISH COLUMBIA CANNED SPRING SALMON PACKED IN CERTAIN CANNERIES IN THE NORTHERN AREA DURING 1938

| Yellow colour, Lovibond units | Interval No. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| 1.5 | 2 | 1 | | | | 1 | | | 1 | | | | | | | | |
| 2.0 | 3 | 1 | 1 | 3 | 3 | 1 | 3 | 1 | 2 | 2 | 1 | 1 | 2 | 3 | | 1 | 3 |
| 2.5 | 6 | 2 | 6 | 8 | 5 | 5 | 2 | 4 | 4 | 6 | 13 | 5 | 10 | 6 | 4 | 1 | 9 |
| 3.0 | 12 | 5 | 8 | 9 | 3 | 1 | 6 | 8 | 7 | 17 | 10 | 4 | 5 | 6 | 2 | 4 | 5 |
| 3.5 | 8 | 2 | 4 | 5 | 2 | 6 | 7 | 4 | 5 | 11 | 10 | 4 | 4 | 1 | 1 | | 3 |
| 4.0 | 1 | 2 | 2 | 5 | 5 | 1 | 2 | 4 | 3 | 5 | 12 | 5 | 2 | 2 | | | 2 |
| 4.5 | 2 | 1 | 5 | 2 | 1 | 2 | 2 | 1 | 2 | 3 | | 1 | 1 | | | | 1 |
| 5.0 | | | | | | | | | | 2 | | | | | | | |
| Total | 34 | 14 | 26 | 32 | 19 | 17 | 22 | 22 | 24 | 46 | 46 | 20 | 24 | 18 | 7 | 6 | 23 |

Elimination of the variations arising from the third factor, however, was somewhat more difficult, because the mean intensities of the red and yellow colours of the muscle tissue of canned spring salmon show considerable seasonal trend. Since the distribution required is the distribution of the red or yellow colour around the line of seasonal trend and the absolute values of the means were of no particular interest, a simple method of combining the data evidently was to adjust the individual distributions occurring in small arbitrarily chosen time intervals to some convenient mean also arbitrarily chosen. For convenience in tabulating, the data were grouped in five-day intervals, the samples in any one time-interval thus being assumed to be derived from salmon of substantially the same biological condition. Time intervals were in all cases determined by the code marks.

Tables I and II show the distributions of the red and yellow colours of the samples of canned spring salmon when the samples are grouped in five-day intervals, while the trends in the means of the distributions given in Tables I and II are illustrated graphically in Fig. 1. From an inspection of the trends

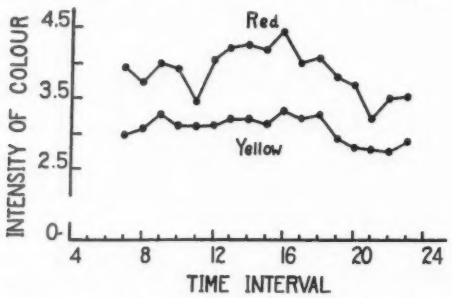


FIG. 1. *Curves showing seasonal trends in means of red and yellow colours of the muscle tissue of canned spring salmon corresponding to the data of Tables I and II.*

in Fig. 1 it will be observed that it is not necessary in all cases to shift the individual distributions to the arbitrary mean, since the distributions can be readily arranged into groups of approximately the same mean intensity of colour. A number of the means of the red colour lie on, or close to, the intensity 4.2. For convenience this was accordingly chosen as the arbitrary mean of the red colour.

To shift the remaining groups to this arbitrary mean we proceed as follows: Let $h$ = grouping interval, $d$ = shift in mean = $m_0 - m_i$, where $m_i$ is the mean of the distribution that it is required to shift to the mean $m_0$. Then for $d < h$ the proportion of frequencies remaining in any one interval is $\frac{h-d}{h}$ The proportion of frequencies in the interval that are to be moved to the next or some succeeding interval is therefore $\frac{1-h-d}{h} = \frac{d}{h}$. These formulae evidently apply also when the absolute value of $d$ is greater than $h$, since, when the distribution is moved an integral number of intervals, its frequencies remain unchanged. To check the calculation the mean of the new distribution is calculated and compared with the arbitrary mean. An illustration of the calculations is shown in Table III.

TABLE III

DETAILS OF CALCULATIONS FOR INTERVAL No. 7 OF TABLE I ILLUSTRATING METHOD OF SHIFTING MEANS OF INDIVIDUAL DISTRIBUTIONS TO ARBITRARY MEAN.

$X$ = intensity of red colour; $f$ = frequency in original distribution; $f_{i+1}$ = frequencies moved to next interval; $f_i$ = frequencies remaining in interval; $F$ = frequency in new distribution.

| $X$ | $f$ | $f_{i+1}$ | $f_i$ | $F$ | $x$ | $Fx$ |
|---|---|---|---|---|---|---|
| 1.5 | 1 | — | 0.48 | 0.48 | −5 | − 2.40 |
| 2.0 | 5 | 0.52 | 2.40 | 2.92 | −4 | −11.68 |
| 2.5 | — | 2.60 | — | 2.60 | −3 | − 7.80 |
| 3.0 | 2 | — | 0.96 | 0.96 | −2 | − 1.92 |
| 3.5 | 4 | 1.04 | 1.92 | 2.96 | −1 | − 2.96 |
| 4.0 | 6 | 2.08 | 2.88 | 4.96 | 0 | — |
| 4.5 | 8 | 3.12 | 3.84 | 6.96 | 1 | 6.96 |
| 5.0 | 4 | 4.16 | 1.92 | 6.08 | 2 | 12.16 |
| 5.5 | 3 | 2.08 | 1.44 | 3.52 | 3 | 10.56 |
| 6.0 | 1 | 1.56 | 0.48 | 2.04 | 4 | 8.16 |
| 6.5 | — | 0.52 | — | 0.52 | 5 | 2.60 |
| Total | 34 | | | 34.00 | | 13.68 |

*$d = 0.26; h - d = 0.24$. Distance from arbitrary origin = 0.402. Mean = 4.201.*

In combining the data of Table I the distributions in intervals No. 13, 14, and 15 were assumed to have the same mean, namely 4.2, while the pairs of intervals 9 and 10, 17 and 18, and 22 and 23 have approximately the means 3.97, 4.04, and 3.51 respectively. Similarly in the case of the yellow colour the mean of each of the intervals 13 and 14, namely 3.2, was taken as the arbitrary origin, while intervals 10, 11, 12, and 15 were assumed to have the same mean 3.14, intervals 16, 17, and 18, the mean 3.25, and intervals 20, 21, and 22, a mean of 2.78.

## Method of Fitting Combined Distributions

Table IV shows the combined or total distributions of the red and yellow colours derived from Tables I and II by combining the distributions occurring in the individual intervals as described in the preceding paragraphs. The general appearance of the distributions of the red and yellow colours of the muscle tissue of canned spring salmon when fewer intervals are combined is shown graphically in Figs. 2 and 3. In all instances, it will be observed, there is evidence that the distribution of the red colour is bimodal. The distribution of the yellow colour, however, is apparently unimodal. In the combined distribution of the red colour given in Table IV the dispersion of the measures around the two modes suggests that the distribution is a mixture of two component normal distributions. Since one of the components is present in smaller quantity than the other and very probably differs in standard deviation from the other, the data correspond to the general case of two component normal distributions.

TABLE IV

COMBINED DISTRIBUTIONS OF RED AND YELLOW COLOURS OF CANNED SPRING SALMON DERIVED FROM THE DATA OF TABLES I AND II

$X$ = colour, $F$ = frequency

| Red colour | | | | Yellow colour | |
|---|---|---|---|---|---|
| $X$ | $F$ | $X$ | $F$ | $X$ | $F$ |
| 1.0 | 0.48 | 5.0 | 78.28 | 1.5 | 4.16 |
| 1.5 | 7.64 | 5.5 | 45.94 | 2.0 | 25.86 |
| 2.0 | 27.02 | 6.0 | 22.66 | 2.5 | 79.86 |
| 2.5 | 36.20 | 6.5 | 5.30 | 3.0 | 110.66 |
| 3.0 | 28.80 | 7.0 | 2.94 | 3.5 | 89.72 |
| 3.5 | 29.96 | 7.5 | 0.90 | 4.0 | 57.70 |
| 4.0 | 43.22 | | | 4.5 | 26.72 |
| 4.5 | 70.66 | | | 5.0 | 5.32 |
| Total | | | 400 | | 400 |

The equations required in solving for the parameters of the component normal distributions in this case are readily derived from the characteristic function (2), namely,

$$\phi(t) = l_1 e^{a_1 t + b_1^2 \frac{t^2}{2}} + l_2 e^{a_2 t + b_2^2 \frac{t^2}{2}},$$

by taking successive derivatives of this function with respect to $t$. On putting $t = 0$ in the results we easily obtain the successive moments of the distribution in terms of the unknown proportions $l_1$ and $l_2$, the means $a_1$ and $a_2$, and the standard deviations $b_1$ and $b_2$ of the component normal distributions. The required moments are as follows:

(1) $\mu_0 = 1 = l_1 + l_2$

(2) $\mu_1 = l_1 a_1 + l_2 a_2$

(3) $\mu_2 = l_1(a_1^2 + b_1^2) + l_2(a_2^2 + b_2^2)$

(4) $\mu_3 = l_1 a_1^3 + 3 l_1 a_1 b_1^2 + l_2 a_2^3 + 3 l_2 a_2 b_2^2$

$$(5) \quad \mu_4 = l_1(a_1^4 + 6a_1^2b_1^2 + 3b_1^4) + l_2(a_2^4 + 6a_2^2b_2^2 + 3b_2^4)$$

$$(6) \quad \mu_5 = l_1(15b_1^4a_1 + 10b_1^2a_1^3 + a_1^4) + l_2(15b_2^4a_2 + 10b_2^2a_2^3 + a_2^4),$$

in which $\mu_0$, $\mu_1$, etc. are the various moments of the combined distribution.

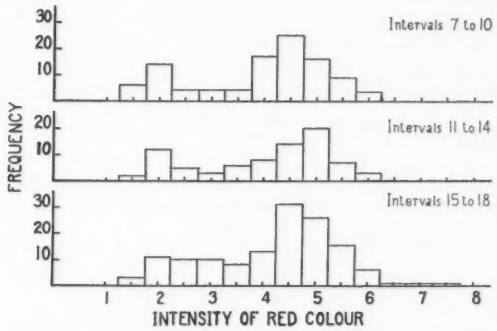


FIG. 2. *Distributions of intensity of the red colour obtained on combining the individual distributions in four successive time intervals.*

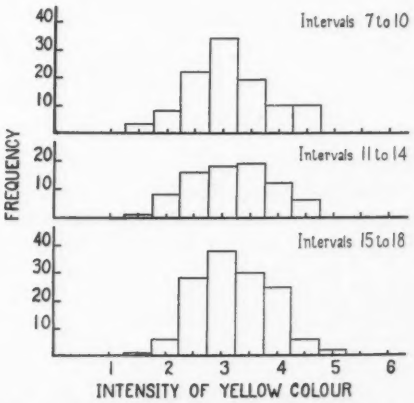


FIG. 3. *Distributions of intensity of the yellow colour obtained on combining the individual distributions in four successive time intervals.*

These equations, which were first derived by Pearson, permit a solution of the general case of a distribution consisting of two component normal distributions. An examination of the combined distribution of the red colour given in Table IV, however, shows that at least half, and possibly more than half, of the distribution in the upper part of the range, that is, a large portion of the red variety in the distribution, may be considered homogeneous. Hence, instead of solving Equations (1) to (6) by the laborious procedure of elimination and solution of the resulting nonic, we may employ the relatively rapid method described by Pearson (5).

Let $d$ be the distance of the stump of the tail from the mean of the truncated portion and $\Sigma$ the standard deviation of the truncated portion about its mean. Also let $\nu_1^1$, $\nu_2^1$ be the first and second moments of the truncated portion around an arbitrary origin $x_0$ units distant from the stump, $\mu_2 = \sigma^2$ the second moment of the normal distribution around its mean, $x$ the distance from the stump to the mean of the curve, $\bar{x}$ the distance of the mean of the truncated portion to the mean of the curve, $n$ the area of the truncated portion, $N$ the whole population, $m_0$, $m_1$, etc., the various normal moment functions, and $i$ the grouping interval. Then as shown by Pearson

$$d = x_0 + \nu_1^1 i = \bar{x} + x$$

$$\Sigma^2 = \nu_2 i^2 = \{\nu_2^1 - (\nu_1^1)^2\} i^2$$

$$\frac{n}{N} = \tfrac{1}{2} + m_0 \tag{7}$$

$$n\bar{x} = N\sigma\left(\frac{1}{\sqrt{2\pi}} - m_1\right) \tag{8}$$

$$n\mu_2 = N\sigma^2(\tfrac{1}{2} + m_2) \tag{9}$$

$$\frac{d}{\sigma} = \frac{\frac{1}{\sqrt{2\pi}} - m_1 + \frac{x}{\sigma}(\frac{1}{2} + m_0)}{\frac{1}{2} + m_0} \tag{10}$$

$$\frac{\Sigma^2}{d^2} = \frac{(\frac{1}{2} + m_2)(\frac{1}{2} + m_0) - \left(\frac{1}{\sqrt{2\pi}} - m_1\right)^2}{\left\{\frac{1}{\sqrt{2\pi}} - m_1 + \frac{x}{\sigma}(\frac{1}{2} + m_0)\right\}^2} \tag{11}$$

The graph of the combined distribution of the red colour given in Table IV suggests that it is safe to assume the truncated portion bounded by the stump 3.75 red units as being very closely homogeneous. Accordingly, the first and second raw moments $\nu_1^1$ and $\nu_2^1$ (Table V) are respectively 2.8752 and 10.0940, $n$ is 269.90, $\Sigma$, 1.8272 and $d$, 1.1876. Hence $\frac{\Sigma^2}{d^2} = \psi_1 = 0.3239$. By trial it is found on applying Equation (11) that this value of $\psi_1$ corresponds

TABLE V

STATISTICAL DATA USED IN CALCULATING THE MEANS AND STANDARD DEVIATIONS OF THE COMPONENT DISTRIBUTIONS IN THE COMBINED DISTRIBUTIONS OF THE RED AND YELLOW COLOURS

*Distribution of red colour*

Arbitrary origin = 4.0; $\mu_1^1 = 0.4020$ half units; $\mu_2 = 6.2229$ half units $^2$; $\mu_1 = 0$; $\mu_2 = 1.5557$ units; Total number of measures = 400.

*Truncated portion of distribution of red colour*

Arbitrary origin = 3.5; stump = 3.75 red units; $n = 269.90$; $\nu_1^1 = 2.8752$ half units; $\nu_2 = 1.8272$ half units$^2$; $d = 1.1876$; $\Sigma^2 = 0.4568$ units.

*Distribution of yellow colour*

Arbitrary origin = 3.0; $\mu_1^1 = 0.4063$ half units; $\mu_2 = 2.0021$ half units$^2$; $\mu_3 = 0.5283$ half units$^3$; $\mu_4 = 10.5560$ half units.$^4$

to a value of $x'\left(=\frac{x}{\sigma}\right)$ lying between 1.2 and 1.3. On calculating the value of $\psi_1$ corresponding to $x' = 1.4$ and applying Stirling's interpolation formula or Formula (iii) in Pearson's "Tables for Statisticians and Biometricians" to the three values 1.2, 1.3, and 1.4 of $x'$ and the corresponding values of $\psi_1$, we find that $x'$ corresponding to the above value of $\psi_1$, namely 0.3239, is 1.2965. Application of the first two terms of the interpolation formula to the values of $\frac{1}{2} + m_0$ and $\frac{1}{\sqrt{2\pi}} - m_1$ corresponding to the same three values of $x'$ then gives the values of $\frac{1}{2} + m_0$ and $\frac{1}{\sqrt{2\pi}} - m_1$ corresponding to the calculated value of $x'$, that is, to $\psi_1 = 0.3239$. The substitution of these values together with the experimental value of $d$ in Equation (10) gives $\frac{d}{\sigma} = 1.4872$, and hence $\sigma = b_1 = 0.7985$. Consequently $x = x'\sigma = 1.0353$; $a_1 = 3.75 + 1.0353 - 4.201 = 0.5843$; $\frac{n}{N} = \frac{1}{2} + m_0 = 0.9026$, and $N = 299.027$.

To find the mean and standard deviation of the other component distribution, that is, of the pale variety of spring salmon, we substitute the parameters of the red component in Equations (1), (2), and (3). The value of $l_1$ is 0.7476, so that $l_2$ is 0.2524. The first moment of the combined distribution $\mu_1$ is zero and the second moment $\mu_2 = 1.557$. Equations (2) and (3) therefore give $a_2 = -1.7304$ and $b_2 = 0.5188$ or, referred to the arbitrary mean, $a_2 = 2.4706$.

As mentioned above, the combined distribution of the yellow colour is unimodal and from an examination of the plotted data appears to be approximately normal. The values of the statistics $k$ and $\beta_2$ of this distribution, however, suggest that this distribution also has been drawn from a non-normal population. The values of $k$ and $\beta_2$ are 0.1865 and 2.6334, respectively. Assuming that the distribution of the yellow colour is approximately normal we have $\sigma_k = \sqrt{\frac{6}{n}} = 0.1225$ and $\sigma_{\beta_2} = \sqrt{\frac{24}{n}} = 0.2450$, so that the calculated values deviate approximately 1.5 standard deviations from the expected values in each case. The values of $k$ and $\beta_2$, however, also deviate in the directions that would be expected if the combined distribution of the yellow colour consists of two component distributions similar to those in the combined distribution of the red colour but differing much less in their means. The chances of drawing two simultaneous values of $k$ and $\beta_2$ from a normal population each deviating 1.5 standard deviations from the expected value is about 1 chance in 60 and the probability that the two values will deviate in this particular direction is one-quarter of this. Hence there is a strong indication that the distribution of the yellow colour is also a mixture of two component populations corresponding to those in the distribution of the red colour.

In view of the close chemical relationship that exists between chlorophyll and the blood pigments and also the close biological relationship that exists

between the red and yellow colours of canned salmon as indicated by the relatively high correlation between the red and yellow colours, it seems safe to assume that the proportions $l_1$ and $l_2$ of the two varieties in the combined distribution of the yellow colour will be the same as those found for the distribution of the red colour. With this assumption the parameters of the component distributions of the yellow colour may then be calculated by means of Equations (2) to (5) from the first four moments of the distribution. The required moments are $\mu_1 = 0$, $\mu_2 = 2.0021$, $\mu_3 = 0.5283$ and $\mu_4 = 10.5560$. Substitution of these values of the moments and the values of $l_1$ and $l_2$, namely 0.7476 and 0.2524 respectively, in Equations (2) to (5) thus gives four equations in the unknown $a_1$, $a_2$, $b_1$, and $b_2$, from which by the elimination of $a_2$, $b_1$, and $b_2$ we obtain the sextic $a_1^6 + 0.05497a_1^3 - 0.05845a_1^2 - 0.00125 = 0$.

The application of Descartes' rule of signs shows immediately that this equation has only one real positive root. Putting $a_1 = 0$ and $a_1 = 1$ in this equation we find that $F(0) = -0.002036$ and $F(1) = 1.0032$ and hence the root lies between 0 and 1. By applying Horner's method the positive root is then found to be $a_1 = 0.4474$.

Since the mean of the combined distribution measured from the arbitrary origin 3.0 was in terms of half the original units as were also the moments, the resulting values of the parameters are also in half units. The mean of the red variety in half units was 0.4062. Accordingly, the mean of the red variety is $3.2031 + 0.2237 = 3.4268$ yellow units. The value of $a_2$ follows at once and is $a_2 = -1.2552$, that is, the mean of the pale variety is 2.5755 yellow units. Similarly, the corresponding standard deviations are $b_1 = 1.2985$, $b_2 = 0.8771$, so that when expressed in whole units the standard deviations of the yellow colours of the red and pale varieties are 0.6493 and 0.4385, respectively.

## Test of Hypothesis

To test the hypothesis as regards the functional forms of the two combined distributions we compare the theoretical distributions with the experimental distributions given in Table IV. This comparison may be effected either graphically or by means of the $\chi^2$ test or by both methods.

Table VI shows the calculated or theoretical values of the ordinates corresponding to given values of the intensity of colour for each of the experimental distributions given in Table IV. These data are illustrated graphically in Figs. 4 and 5. From an inspection of these figures it will be observed that the theoretical distributions describe with a high degree of accuracy the experimental data.

This conclusion is also confirmed by the $\chi^2$ test. Tables VII and VIII show the observed and theoretical frequencies falling into the various classes in the combined distributions of the red and yellow colours. The number of classes and the value of $\chi^2$ for the combined distribution of the red colour are respectively 11 and 2.080, and the corresponding figures for the combined

TABLE VI

ORDINATES OF THE COMPONENT NORMAL DISTRIBUTIONS AND OF THE RESULTING COMBINED DISTRIBUTIONS OF THE RED AND YELLOW COLOURS CORRESPONDING TO THE DATA OF TABLE IV

| Colour | Red | | | Yellow | | |
|---|---|---|---|---|---|---|
| | Pale variety | Red variety | Combined | Pale variety | Red variety | Combined |
| 1.0 | 0.72 | | 0.72 | 0.07 | 0.09 | 0.16 |
| 1.5 | 6.77 | | 6.77 | 2.30 | 1.17 | 3.47 |
| 2.0 | 26.08 | 0.19 | 26.27 | 19.54 | 8.48 | 28.02 |
| 2.5 | 38.79 | 1.25 | 40.04 | 45.29 | 33.88 | 79.17 |
| 3.0 | 23.54 | 6.29 | 29.83 | 29.20 | 74.73 | 103.93 |
| 3.5 | 5.51 | 21.08 | 26.59 | 5.16 | 91.34 | 96.50 |
| 4.0 | 0.52 | 46.34 | 46.86 | 0.25 | 62.53 | 62.78 |
| 4.5 | | 70.41 | 70.41 | | 23.65 | 23.65 |
| 5.0 | | 72.38 | 72.38 | | 4.95 | 4.95 |
| 5.5 | | 50.51 | 50.51 | | 0.58 | 0.58 |
| 6.0 | | 23.59 | 23.59 | | | |
| 6.5 | | 7.69 | 7.69 | | | |
| 7.0 | | 1.63 | 1.63 | | | |
| 7.5 | | 0.24 | 0.24 | | | |

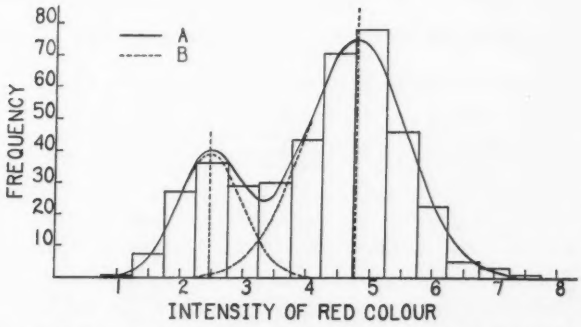


FIG. 4. *Graphical illustration of the combined distribution of the intensity of the red colour showing experimental and theoretical distributions. A = combined distribution; B = component distributions.*

distribution of the yellow colour are 8 and 2.257. Since the observed data in this case were used in calculating the values of the parameters in the theoretical distribution, the number of classes (7) must be diminished by six in each case in computing the number of degrees of freedom, since this is the number of conditions employed in calculating the values of the parameters. The number of degrees of freedom of the distributions of the red and yellow colours are accordingly 5 and 2 respectively. Reference to Fisher's table (3) shows that both these values of $\chi^2$ might very easily have arisen through sampling fluctuation. The probability associated with the value 2.080 is approximately $P = 0.85$, while that associated with the value

2.257 is about $P = 0.50$. There is thus no reason to suspect the hypothesis tested and the theoretical distribution must, therefore, be regarded as fitting very closely the experimental data.

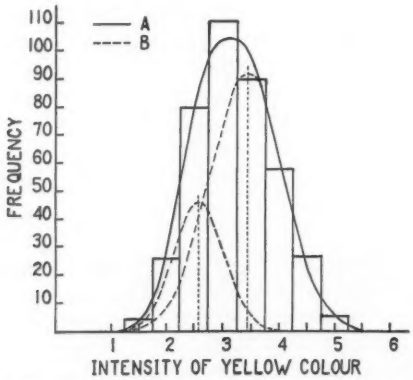


FIG. 5. *Graphical illustration of the combined distribution of the intensity of the yellow colour showing experimental and theoretical distributions. A = combined distribution; B = component distributions.*

TABLE VII

COMPARISON OF OBSERVED AND THEORETICAL FREQUENCIES IN THE COMBINED DISTRIBUTION OF THE RED COLOUR

| Colour | Obs'd $f$ $(m + x)$ | Calc'd $f$ $(m)$ | $x$ | $\frac{x^2}{m}$ |
|---|---|---|---|---|
| 0.5<br>1.0<br>1.5 | 8.12 | 8.32 | −0.20 | 0.005 |
| 2.0 | 27.02 | 25.73 | 1.29 | 0.065 |
| 2.5 | 36.20 | 38.74 | −2.54 | 0.166 |
| 3.0 | 28.80 | 29.62 | −0.82 | 0.023 |
| 3.5 | 29.96 | 27.00 | 2.96 | 0.324 |
| 4.0 | 43.22 | 46.68 | −3.46 | 0.256 |
| 4.5 | 70.66 | 69.12 | 1.54 | 0.034 |
| 5.0 | 78.28 | 70.96 | 7.32 | 0.755 |
| 5.5 | 45.94 | 49.88 | −3.94 | 0.311 |
| 6.0 | 22.66 | 23.98 | −1.32 | 0.073 |
| 6.5<br>7.0<br>7.5<br>8.0 | 9.14 | 9.96 | −0.82 | 0.068 |
| Total | 400.00 | 399.99 | | 2.080 |

## Concluding Remarks

Sheppard's corrections for grouping, it will be observed, were not applied in the foregoing calculations, but instead, the raw moments were substituted directly into the equations. Shewhart (7) gives a number of reasons why it may not be advisable in all cases to apply such corrections. In the present

TABLE VIII

COMPARISON OF OBSERVED AND THEORETICAL FREQUENCIES IN THE COMBINED DISTRIBUTION OF THE YELLOW COLOUR

| Colour | Obs'd $f$ $(m + x)$ | Calc'd $f$ $(m)$ | $x$ | $\frac{x^2}{m}$ |
|---|---|---|---|---|
| 1.0<br>1.5 | 4.16 | 4.47 | −0.31 | 0.021 |
| 2.0 | 25.86 | 29.08 | −3.22 | 0.356 |
| 2.5 | 79.86 | 76.97 | 2.89 | 0.108 |
| 3.0 | 110.66 | 101.61 | 9.05 | 0.806 |
| 3.5 | 89.72 | 95.00 | −5.28 | 0.293 |
| 4.0 | 57.70 | 62.24 | −4.54 | 0.331 |
| 4.5 | 26.72 | 24.42 | 2.30 | 0.217 |
| 5.0<br>5.5<br>6.0 | 5.32 | 6.20 | −0.88 | 0.125 |
| Total | 400.00 | 399.99 | | 2.257 |

instance the most cogent reason for not applying the corrections would appear to be the relatively small sample size (269.9) employed in calculating the second moment of the truncated portion of the distribution of the red colour. If $h$ is the grouping interval, Sheppard's correction for the second moment is $-\frac{h^2}{12}$. In this instance, however, the standard deviation of the second moment is (4, 6).

$$\sigma_{\mu_2} = \sqrt{\frac{\mu_4 - \mu_2^2}{n}},$$

where $n$ is the sample size. The values of $\mu_4$ and $\mu_2$ of the truncated part expressed in half units were 11.4430 and 1.8272 respectively. Hence, in half units $\sigma_{\mu_2} = 0.1733$ or $\sigma_{\mu_2} = 0.04332$ red units. Sheppard's correction, namely, 0.02083, is thus less than half the standard error of the second moment, so that it would be a useless refinement to apply the correction in this case.

Another point that should be noted in the above calculations is the approximation employed in combining the individual distributions in the various time intervals. The means of the individual distributions have been used as estimates of the true means of the red and yellow colours corresponding to the respective time intervals. This procedure will introduce slight errors into the final results owing to sampling fluctuations in the means. Here again, however, the simpler procedure would seem to be preferable in this case to the questionable refinement of fitting the trends with meaningless third or fourth degree power series.

Finally, it is interesting to note that the difference in the means of the red colour for the two varieties is $4.7853 - 2.4706 = 2.3147$ and the difference in the means of the yellow colour, $3.4268 - 2.5755 = 0.8513$. The absolute values of the means, of course, are arbitrary. Also, the two varieties of spring salmon occur very nearly in the proportions of 1 to 3, that is, very nearly one-

quarter of all the samples consist of the pale variety while the remaining three-quarters is made up of the red variety of this species.

The value 0.7476, it will be observed, is in complete accord with the hypothesis that the true proportion is 0.7500. For, if a sample of 400 were drawn from a population in which the true proportion of the red variety was 0.7500, the standard deviation of the observed proportion would be

$$\sigma_p = \sqrt{\frac{pq}{n}} = 0.02165.$$

Consequently, the observed value 0.7476 differs only slightly more than one-tenth standard deviation from the hypothetical value 0.7500 and there are accordingly no grounds for concluding that the true proportion is not 0.7500.

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# PARASITES OF FRESHWATER FISH

## III. FURTHER STUDIES ON THE INTERNAL TREMATODES OF FISH IN THE CENTRAL ST. LAWRENCE WATERSHED[1]

By M. J. Miller[2]


### Abstract

Three new species of internal trematodes, *Plagioporus serratus* sp. nov., *Phyllodistomum lysteri* sp. nov., and *Parastiotrema ottawanensis* gen. et sp. nov., are described from Canadian freshwater fish. *Plagioporus serotinus* Stafford, 1904, is redescribed, and the genus *Caudotestis* Yamaguti, 1934, is reduced to synonymity with *Plagioporus* Stafford, 1904. *Anallocreadium pearsei* Hunter and Bangham, 1932, is considered synonymous with *A. armatum* McCallum, 1895. The genus *Bunoderina* Miller, 1936, is reduced to synonymity with *Bunodera* Railliet, 1896.


### Introduction

This paper, the third in a series on the parasites of freshwater fish in Canada, presents the results of the survey that has been continued since the first paper (6) appeared; it deals with non-commercial as well as commercial fish. All the fish were taken from the Ottawa River near its confluence with the St. Lawrence River, at Ste. Anne de Bellevue, Quebec.

### Species Recovered

Family Allocreadiidae

Genus *Plagioporus* Stafford, 1904

Synonyms: *Lebouria* Nicoll, 1909,

*Caudotestis* Yamaguti, 1934.

Stafford created the genus *Plagioporus* to include *P. serotinus*, a trematode he recovered from the intestines of *Moxostomata macrolepidotum* (red horse sucker). Nicoll (9) erected the genus *Lebouria* for his species *L. idonea* and included in the genus a trematode described by Linton from *Bairdiella chrysura* and named by Nicoll *L. abducta*. Price (12) pointed out that *Lebouria* was congeneric with *Plagioporus* and, as the latter genus has priority, he transferred the species of *Lebouria* to the genus *Plagioporus*, thus reducing *Lebouria* to synonymity. The subgenus *Caudotestis* (Issaitschikow, 1928) was raised to generic rank by Yamaguti (16) to include those species of *Plagioporus* having the intestinal caeca terminating at the level of the testes, and the vitellaria usually not extending into the post-testicular body region. However, these two characters are not sufficiently well defined in the species of the two genera, which show all the intergradations. Thus, *P. cooperi* has the caeca terminating at the level of the testes, but the post-testicular vitellaria

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are numerous, and meet medially. It is apparent, therefore, that *Caudotestis*, as it is now defined, cannot be retained as a separate genus and must be considered as a synonym of *Plagioporus*.

Mueller (8) stated that Stafford's species *P. serotinus* could not be retained because it was not recognizable. However, the type specimen has been recovered. Furthermore, in the intestines of the red horse and common sucker have been found additional specimens which differ only in that they are of a larger size in the former host.

*Plagioporus serotinus* Stafford, 1904
(Figs. 2 and 3)

HOSTS: *Moxostoma aureolum* (red horse sucker)
*Catostomus commersonii* (common sucker)

The worms of this species are small, narrow forms, broadest in the middle and tapering gradually to both extremities. The cuticula is smooth. They measure from 1.0 to 1.4 mm. in length in the common sucker, whereas specimens from the red horse sucker measure up to 2.2 mm. in length. They are approximately one-fourth as broad as long. The oral sucker is terminal,

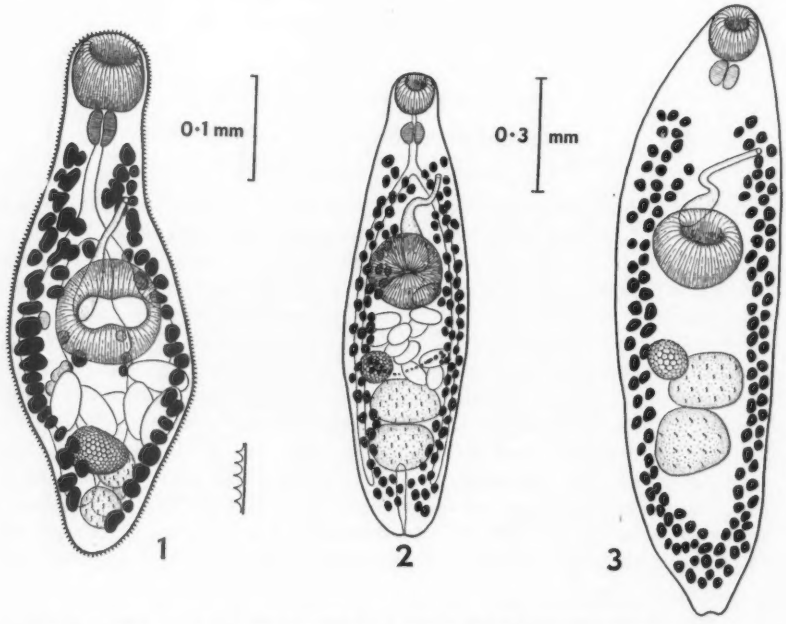



FIG. 1. *Plagioporus serratus, ventral view.* FIG. 2. *P. serotinus, ventral view, specimen from common sucker.* FIG. 3. *P. serotinus, ventral view, specimen from Stafford's collection from red horse sucker.*

subspherical, and measures from 0.08 to 0.14 mm. in diameter. The acetabulum is situated two-fifths of the body length from the anterior tip, and is approximately twice the diameter of the oral sucker. There is a short pre-pharynx and a well developed pharynx whose length is about half the diameter of the oral sucker. The oesophagus is from once to twice the length of the pharynx. The intestinal caeca extend to within a short distance of the posterior extremity. The testes are in tandem arrangement, one immediately behind the other, in the middle of the posterior half of the body. Occasionally the anterior testis is displaced slightly to the left. The testes are smooth-margined and usually somewhat flattened in the antero-posterior axis. The ovary is spherical and is situated immediately anterior of the anterior testis, on the right side of the body. The vitellaria extend laterally from near the pharynx to within a short distance from the posterior tip; they meet in the post-testicular body space. The uterus extends anteriorly from the ovary to the genital pore, which is situated at the level of the intestinal bifurcation, on the left side of the body. The cirrus pouch overlaps the acetabulum one-quarter to one-half its diameter. The excretory bladder is small and sac-like. The eggs measure 0.07 to 0.09 mm. by 0.05 to 0.06 mm.

*Plagioporus serratus* sp. nov.
(Fig. 1)

HOST: *Hyodon tergisus* (moon-eye)

Members of this species occur in the gall bladder of their host. They are very small, somewhat spindle-shaped flukes, tapering rather abruptly at the posterior tip and narrowing out to a broad neck at the anterior end. The cuticula is characteristically extended to form conspicuous broad-based spines, which cover the entire body. Mature specimens measure from 0.41 to 0.51 mm. in length by 0.14 to 0.18 mm. in width at the widest point, which is usually the region of the acetabulum. The oral sucker is terminal, subspherical and measures from 0.076 to 0.080 mm. in diameter. The acetabulum is situated about the middle of the body. It is subspherical and somewhat larger than the oral sucker, measuring from 0.09 to 0.11 mm. in diameter. There is no obvious pre-pharynx. The pharynx is prominent and is about half as long as the diameter of the oral sucker. The oesophagus is from once to twice the length of the pharynx. The broad intestinal crura extend to within a short distance of the posterior extremity. The testes are obliquely arranged near the posterior tip of the body; the posterior testis is on the mid-line and the anterior testis is immediately to the left of it. They are approximately spherical in outline with smooth edges, and measure from 0.042 to 0.046 mm. in diameter. The ovary is ovate in outline with the long axis in the lateral plane; it is larger than the testes and is situated on the mid-line partly overlapping the anterior testis. The vitellaria extend laterally from the posterior end of the pharynx to the posterior testis. The uterus passes anteriorly to the genital pore situated on the left side of the body slightly

anterior to the level of the intestinal bifurcation. The cirrus pouch overlaps the acetabulum to about half its diameter. The eggs are comparatively large, measuring 0.067 by 0.038 mm. There are usually from four to six eggs in the uterus.

*Plagioporus serratus* differs from all previously described species of this genus by its extremely small size, and the spinose extensions of its cuticula.

As far as can be determined eight species of *Plagioporus* have been reported from American fishes. Included in this number is *Plagioporus (Lebouria) abducta* (Nicoll, 1909), a parasite described by Linton (1904) from *Bairdiella chrysura*. However, *P. abducta* cannot be retained in the genus *Plagioporus*, as the median position of its genital pore violates the concepts of the genus. A key to the American species of *Plagioporus* is presented below.

1. Cuticula with large spinose extensions; small worms under 0.6 mm. in length ..... *P. serratus* sp. nov.
   Cuticula smooth, without spinose extensions ..... 2.
2. Body strongly fusiform; acetabulum broadly ovate with the long axis in the lateral plane, over twice the size of the oral sucker.
   *P. fusiformis* Price, 1934.
   Body not strongly fusiform; acetabulum more or less spherical, not over twice the diameter of the oral sucker ..... 3.
3. Vitellarian follicles numerous in post-testicular body region ..... 4.
   Vitellarian follicles not numerous in post-testicular body region, extending just beyond the testes; testes near posterior tip of body.
   *P. sinitsini* Mueller, 1934.
4. Intestinal crura not extending posterior of the posterior testis.
   *P. cooperia* Hunter and Bangham
   Intestinal crura extending posterior of the posterior testis ..... 5.
5. Vitellarian follicles not extending anterior of the acetabulum.
   *P. lepomis* Dobrovolny, 1939.
   Vitellarian follicles extending anterior of the acetabulum ..... 6.
6. Vitellarian follicles anterior of the acetabulum for the most part confined laterally; acetabulum approximately twice the diameter of the oral sucker ..... *P. serotinus* Stafford, 1904.
   Vitellarian follicles anterior of the acetabulum meeting medially; acetabulum considerably less than twice the diameter of the oral sucker ..... *P. crassigula* Linton, 1911.

*Anallocreadium armatum* McCallum, 1895

(Fig. 10)

Synonym: *Anallocreadium pearsei* Hunter and Bangham, 1932.

HOSTS: *Ameiurus nebulosus* (bullhead).
*Eupomotis gibbosus* (sunfish).

This parasite was found rather commonly in the intestine of sunfish, but only five specimens were recovered from the bullhead. All specimens with the exception of one from the sunfish and two from the bullheads contained no eggs.

The mature specimens measure from 1.23 to 2.31 mm. in length by 0.5 to 0.8 mm. wide. They have rounded extremities with the posterior end broader. Spines can be seen on the cuticula of some specimens but only with difficulty. The oral sucker is subspherical. There is a well developed pre-pharynx, a pharynx which measures slightly less than half the diameter of the oral sucker, and a short oesophagus which forks about one-third of the distance between the pharynx and the acetabulum. The intestinal caeca extend well into the posterior portion of the body. The acetabulum is slightly larger than the oral sucker, and is situated in the second quarter of the body. The testes are in tandem arrangement in the third quarter of the body. they are broadly ovate to spherical in outline with smooth margins and may be smaller or larger than the acetabulum. The ovary is small, spherical in outline and situated on the right side of the body immediately anterior of the anterior testis. The vitellaria extend laterally from the posterior margin of the acetabulum to the posterior tip of the body filling in the post-testicular space. The uterus passes anterior from the ovary to the genital pore situated on the mid-line immediately anterior of the acetabulum. Measurements of an average specimen are as follows: length 2.06 mm., width 0.59 mm., oral sucker 0.22 mm. in diameter, acetabulum 0.25 mm. in diameter, ovary 0.13 mm. in diameter, anterior testis 0.24 by 0.31 mm., posterior testis 0.26 by 0.31 mm., egg 0.09 to 0.11 by 0.06 mm.

Hunter and Bangham (4) created the species *Anallocreadium pearsei* for a trematode in the intestine of *Aplodinotus grunniens*. Pearse (11) reported a species of *Anallocreadium* from the same host and from *Eupomotis gibbosus* which he called *A. armatum* but which Hunter and Bangham consider to be *A. pearsei*. The characters used by these authors to separate *A. pearsei* from *A. armatum* are the nature of the margin of the testes, and the comparative sizes of the suckers, of the pharynx, and acetabulum, and of the testes and acetabulum. According to them *A. pearsei* has the oral sucker only slightly smaller than the acetabulum, while *A. armatum* has an acetabulum twice the size of the oral sucker. They consider the testes to be always larger than the acetabulum in *A. pearsei* and usually smaller in *A. armatum*. They state the length of the pharynx to be about one-third the diameter of the acetabulum in *A. armatum* and one-half the diameter in *A. pearsei*. Finally, they consider the testes to have smooth margins in *A. armatum* and lobed margins in *A. pearsei*.

The specimens recovered in the present survey show characters of both species. The oral sucker is only slightly smaller than the acetabulum which is characteristic of *A. pearsei*, but the testes are smooth-margined, a characteristic of *A. armatum*. The length of the pharynx varies from one-third to one-half the diameter of the acetabulum, and the testes range in size from

smaller, to considerably larger than, the acetabulum. It appears, therefore, that there is but one valid species of *Anallocreadium*, and that *A. pearsei* must be considered a synonym of *A. armatum*.

*Bunodera eucaliae* (Miller, 1936)

Synonym: *Bunoderina eucaliae* Miller, 1936

HOST: *Eucalia inconstans* (stickleback).

This parasite, originally referred to a new genus by the author because of the character of its uterus which is tubular rather than sacculate, resembles *Bunodera sacculata* Van Cleave and Mueller (15) in so many characters that it seems more consistent to place it in the same genus. The tubular uterus in *B. eucaliae* probably points out the relationship of *Bunodera* and *Crepidostomum* and may indicate the direction of the evolutionary trend.

Three species of *Bunodera* have been reported from North America. They can be distinguished as follows:

1. Vitellaria extending laterally from anterior of the acetabulum to near the posterior tip of the body ..... *B. luciopercae* Müller, 1776.
   Vitellaria extending laterally from anterior of the acetabulum to about the middle of the body length ..... 2.
2. Vitellaria not extending to the level of the testes; acetabulum larger than oral sucker; mature forms with tubular uterus .. *B. eucaliae* Miller, 1936.
   Vitellaria extending to the level of the testes; acetabulum not larger than oral sucker; mature forms with sacculate uterus
   *B. sacculata* Van Cleave and Mueller, 1934.

FAMILY PLAGIORCHIDAE

*Parastiotrema ottawanensis* gen. et. sp. nov.

(Figs. 4 and 5)

HOST: *Ictalurus punctatus* (channel catfish)

Only three specimens of this small trematode were recovered from the intestine of the channel catfish. One of the specimens was contracted to such an extent that it could not be studied.

Members of this species are small muscular worms, pointed at the posterior tip and more or less rounded anteriorly. The middle part of the body is parallel-sided. The cuticula is covered with very fine spines which are more prominent anteriorly and gradually disappear toward the posterior part of the body. One of the specimens is longer and narrower than the other, measuring 0.72 by 0.22 mm.; the other measures 0.58 by 0.27 mm. The oral sucker is spherical to subspherical in outline; in one specimen it measures 0.092 and in the other 0.108 mm. in diameter. The acetabulum is approximately the same size as the oral sucker. It is situated in the first or second quarter of the body, depending on the state of contraction of the body. The pharynx is about half as long as the oral sucker. There is no apparent pre-

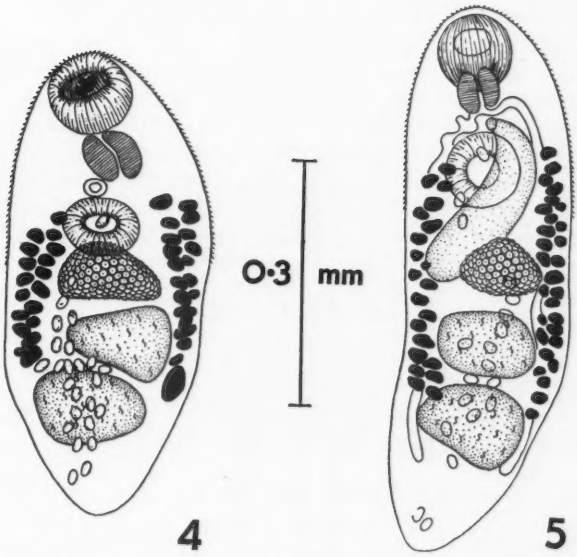



FIG. 4. *Parastiotrema ottawanensis, ventral view.* FIG. 5. *P. ottawanensis, dorsal view.*

pharynx nor does there appear to be an oesophagus. The intestinal crura lead to the posterior border of the posterior testis. The testes are comparatively large, with smooth or slightly irregular margins; they are roughly triangular in shape with the long axis in the lateral plane. They measure up to 0.08 mm. in width, and are in tandem arrangement with the anterior displaced slightly to the left. The ovary is situated between the anterior testis and the acetabulum. It is triangular in shape and somewhat smaller than the testes. The uterus passes back to the posterior tip of the body. The vitellaria extend laterally from the anterior margin of the acetabulum to the posterior margin of the posterior testis. The genital pore is situated on the mid-line immediately anterior of the acetabulum. The cirrus pouch is long and broad extending posteriorly to overlap the ovary. The eggs measure 0.025 by 0.017 mm.

The genus *Parastiotrema* appears to be closely related to *Astiotrema* Looss, 1900, and *Alloglossidium* Mueller, 1930. Like *Astiotrema* it has a very large cirrus pouch which differs, however, in that, unlike the cirrus sac in that species, it does not have the terminal end particularly thin. The large triangular shaped genital organs are quite distinct from those exhibited by the species of *Astiotrema* as is the character of the alimentary tract. It differs from *Alloglossidium* mainly in the character of the alimentary tract, and in the shape and size of the testes and ovary.

*Generic Diagnosis*

Plagiorchiinae; small parallel-sided forms, more or less pointed at the posterior extremity, and rounded at the anterior end. Cuticula with minute spines; alimentary tract with well developed oral sucker and pharynx, but with no apparent pre-pharynx or oesophagus. Testes and ovary large, roughly triangular in outline; testes in the posterior half of the body in tandem arrangement; ovary anterior of the anterior testis; uterus extending back to the posterior tip of the body. Cirrus sac very large and broad, extending back to the posterior margin of the ovary; vitellaria laterally arranged from the acetabulum to the testis; occurring in the intestine of fishes.

*Alloglossidium geminus* Mueller, 1930

(Fig. 9)

HOST: *Ameiurus nebulosus* (bullhead).

Specimens of this species are common in the intestine of the bullhead in this vicinity. They range from 0.68 to 1.27 mm. in length.

*Alloglossidium corti* Lamont, 1921

(Figs. 7 and 8)

HOST: *Ictalurus punctatus* (channel catfish).

This species was very commonly recovered from the intestine of the channel catfish. Mature specimens ranged from 0.53 to over 2.0 mm. in length. The smaller specimens have the genital organs and the acetabulum situated proportionately further back in the body, and the body is comparatively wider. The presence of intermediate forms, however, shows the two types to be of the same species. As pointed out by van Cleave and Mueller (15) this species can be separated from *A. geminus* by the character of the vitellaria which extend anterior of the acetabulum in *A. corti*, and not anterior of the anterior margin of the acetabulum in *A. geminus*.

FAMILY HETEROPHYIDAE

*Cryptogonimus chyli* Osborn, 1903

(Fig. 6)

HOSTS: *Ambloplites rupestris* (rock bass)
*Micropterus dolomieu* (small-mouth black bass)

Only a few specimens of this species were obtained from the above mentioned hosts.

FAMILY AZYGIIDAE

*Azygia angusticauda* Stafford, 1904

HOSTS: *Stizostedion vitreum* (doré)
*Micropterus dolomieu* (small mouth black bass)

Three specimens of this species were recovered, one from the intestine of the bass, and the other two from the intestine of the doré. The specimen

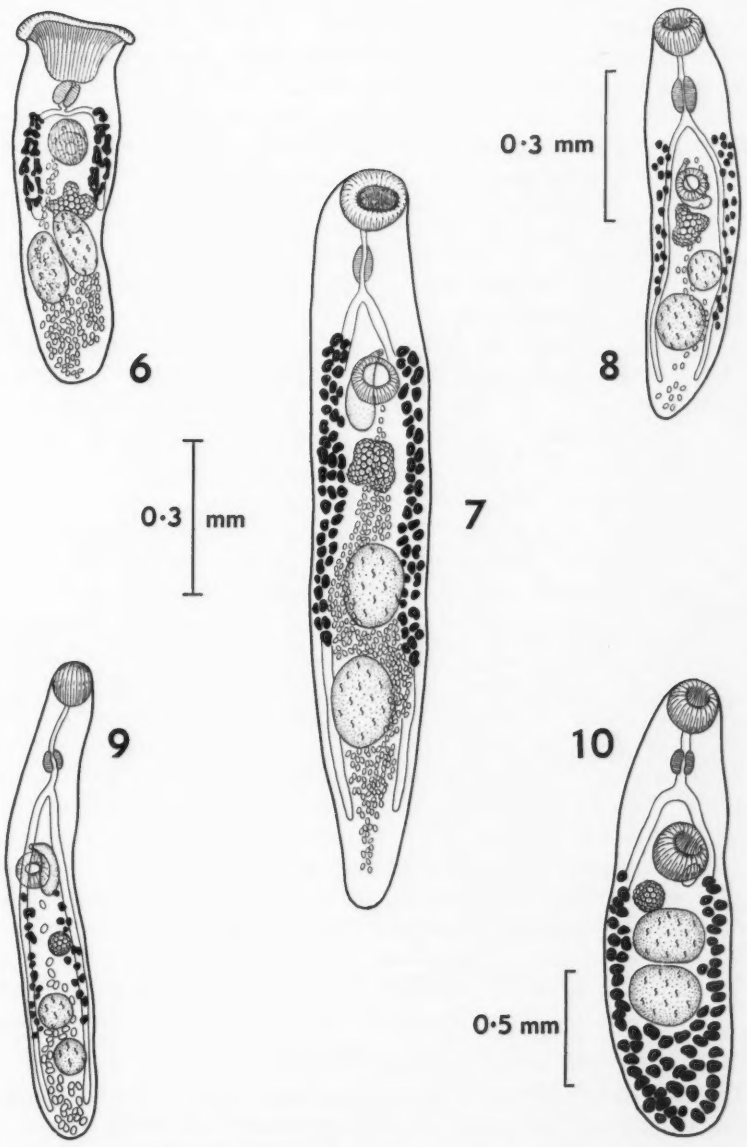


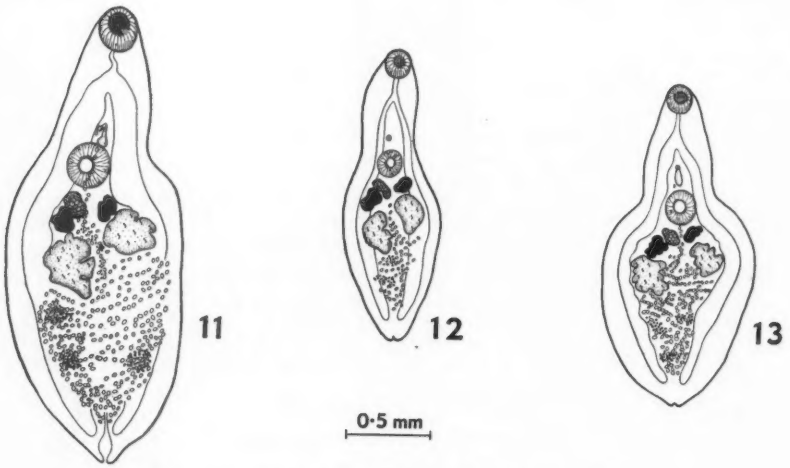

FIG. 6. *Cryptogonimus chyli, dorsal view.* FIGS. 7 AND 8. *Alloglossidium corti, ventral views.* FIG. 9. *A. geminus, dorsal view.* FIG. 10. *Anallocreadium armatum, dorsal view.*

from the bass measured slightly less than 2.0 mm. in length, while those from the doré measured 11 and 12 mm. respectively. Van Cleave and Mueller (15) reported similar large specimens from the doré and it is possible that they may represent a distinct subspecies.

## Family Gorgoderidae

### Genus *Phyllodistomum* Braun, 1899

As far as can be determined, up to the present time seven species of *Phyllodistomum* have been reported from American fishes. During the present survey, a species of *Phyllodistomum* was recovered from the urinary tubules of *Catostomus commersonii* and upon careful examination it proved to be heretofore undescribed. The name *P. lysteri* is proposed for this species in honour of the late L. L. Lyster by whom the first specimens of this species were collected.




Figs. 11, 12, 13. *Phyllodistomum lysteri, ventral view.*

*Phyllodistomum lysteri* sp. nov.

(Figs. 11, 12, and 13)

Host: *Catostomus commersonii* (common sucker)

The body of this species is flattened and made up of a narrow anterior region and a broad posterior part; the approximate ratio of the anterior to the posterior part of the body is as 1 : 2. The posterior end of the body is obtusely pointed and may show a small notch. The cuticula is smooth, and the body margins do not show any crinkling. Specimens range from

1.75 to 3.2 mm. in length by 0.5 to 1.0 mm. in width. The oral sucker is situated at the junction of the narrow and wider portions of the body; it may be the same size or very slightly larger than the oral sucker, measuring from 0.20 to 0.26 mm. in diameter. There is no pharynx. The oesophagus is approximately the same length as the oral sucker; it divides into two broad intestinal crura which extend to within a short distance of the posterior tip of the body. There are two large testes with irregularly lobed margins, situated in the middle third of the body. They are obliquely arranged with the left testis usually more anterior than the right, although this arrangement may be reversed. The lobed ovary is about one-third the size of the testes and is situated immediately anterior of the more posterior testis. The uterus fills the posterior part of the body between the intestinal crura, finally leading to the genital pore which is situated on the mid-line, a short distance above the acetabulum. There are two solid, lobed, vitellarian bodies, lying between the acetabulum and the genitalia, in tandem arrangement. The uterus contains what appear to be two types of eggs. One is a typical egg with an oval outline and a well developed egg shell, measuring 0.031 to 0.036 mm. by 0.018 to 0.021 mm. The other is spherical in outline and does not show a well developed egg shell. It is larger than the more typical egg measuring 0.032 to 0.052 mm. in diameter, and stains readily with alum carmine. The uterus of every mature specimen shows these two types of eggs.

*Phyllodistomum lysteri* is readily distinguishable from *P. pearsei*, *P. carolini*, *P. staffordi*, *P. lacustri*, and *P. fausti* by the fact that the oral and ventral suckers are not obviously different in size in this species, while they do show obvious difference in size in the last five species. It is further differentiated from *P. fausti* by not having the post-acetabular body widest in the middle and by the space between the testis, from *P. staffordi* and *P. carolini* by not having the post-acetabular part of the body discoidal, and from *P. lacustri* by having a smooth cuticula. It most closely resembles *P. superbum* and *P. folium*. However, it is distinguished from these two species by the more anterior position of its gonads and by the ratio of the pre- to the post-acetabular regions of the body which in *P. lysteri* is about 1 : 2, whereas in the *P. superbum* and *P. folium* it is 1 : 1.25. Furthermore it can be differentiated from *P. folium* by its larger size, and from *P. superbum* by the fact that the body margins are not crinkled.

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# COLOUR OF MEAT

## III. AN IMPROVED COLOUR COMPARATOR FOR SOLIDS[1]

By C. A. Winkler,[2] W. H. Cook,[3] and E. A. Rooke[4]


### Abstract

A photoelectric colour comparator, previously described (Can. J. Research, D, 17: 1-7. 1939) has been improved to permit greater precision and more rapid operation. Measurements on meat indicate that the degree of precision attainable with the new comparator is determined primarily by sampling, rather than instrumental, errors.


### Introduction

An objective colour comparator suitable for estimating the colour of solids was described in the first paper of this series (1). The results obtained with this instrument in subsequent investigations on the colour (2, 4) and colour stability of bacon (3, 5) led to the construction of an improved model. Enquiries requesting a more detailed description and other information regarding the earlier apparatus prompted the preparation of this article describing the new instrument.

The new apparatus, like the old, is based on the measurement of the amount of each main component of white light, namely blue, green, and red, scattered by the test sample and expressed as a percentage of that scattered by a standard white surface. Such measurements naturally yield less information than those made with a spectrophotometer, but have the advantage that they can be made more rapidly. This feature enables measurements to be made on labile materials without appreciable change, and facilitates making a sufficient number of measurements to estimate the variability present in such biological materials as meat. The equipment was originally designed to place the subjective visual estimates of colour and colour stability on an objective basis. Subsequent papers (3, 4, 5) have shown that not only was the older instrument capable of detecting significant differences in the colour of different samples, but it was also possible, by statistical studies of a large number of measurements, to relate the scatter of the individual colour components to the composition of the bacon with respect to certain constituents.

### Description of Apparatus

The new instrument is fundamentally the same as the earlier model. The most important changes are: baffle plates to minimize internal reflections; diaphragm for controlling light intensity, thus enabling the light source to be

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operated at fixed voltage and avoiding major change in colour quality; a more sensitive photo-cell, and a more sensitive galvanometer. Mechanically the apparatus was much improved, permitting greater accuracy and more rapid operation.

A photograph of the apparatus is shown in Fig. 1 and a diagrammatic sketch in Fig. 2. It is impossible to give complete details of construction in this article but the information contained in the figures and the following brief description are considered adequate to permit the construction of a comparable instrument wherever the necessary facilities are available.

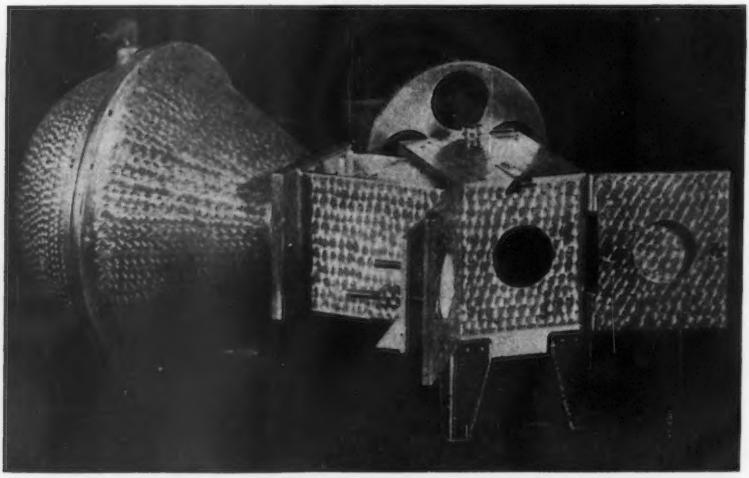

FIG. 1. *Colour comparator showing general arrangement, lamp housing, reference and test sample holders, and selective filter holder.*

The two sections of the lamp housing, $A$, and the adapter section, $K$, are dural castings with drilled and tapped flanges for attachment. The remainder of the apparatus is constructed from flat or angle aluminium fastened with screws to facilitate assembly.

The two sections of the lamp housing are water jacketed, and a continuous circulation of cooling water is maintained during operation. The hemispherical portion at the back is polished on the inside. A semi-circular opening is provided between the two halves of the lamp housing to accommodate a No. 2 photo-flood bulb. The walls of this portion of the housing are thicker and shaped to permit the attachment of an aluminium shield, supporting the lamp socket, to the housing with screws. As the photo-flood bulb must be screwed into the socket from the inside, the two sections of the housing must be separated when the bulb is changed, but as this is seldom necessary it is not a troublesome operation.

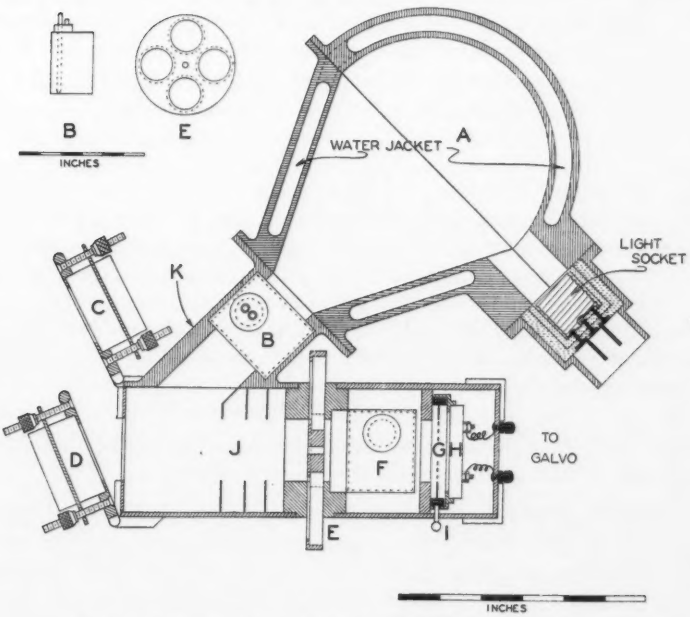


FIG. 2. *Diagrammatic sketch of colour comparator (plan view).*

The adapter section, *K*, is of rectangular cross section and flanged for attachment to the lamp housing. The end furthest removed from the housing is shaped to permit attachment to the metering portion at an angle of 45°. An oval opening, 1.5 in. high and 1.06 in. wide, is provided through the centre of this block, to allow the incident light at an angle of 45° to fall uniformly on the 1½ in. diameter circular opening containing the test sample. A rectangular recess, open at the top, is provided at right angles to the path of the light to accommodate the water cell, *B*, for removing part of the infra-red radiation from the incident light beam. This cell may be glass but a plastic (plexiglass) has been found more convenient. The cap for this cell is provided with two tubes for the circulation of cooling water (tap) during the course of the measurements.

The reference standard and test sample holders, *C* and *D*, consist of removable doors hinged on opposite sides of the rectangular part of the instrument. Each door consists of two plates arranged to hold the sample between them by clamping. The outer plate is solid, lightproof, and moves back or forth on the clamping screws. The inner plate is fixed and has a centrally drilled hole 1½ in. in diameter to expose the material. A similarly drilled plate member, darkened on the inside, forms the back of the instrument. The test sample is usually placed on an aluminium plate, or in a short cylinder with a locating pin if the exact test position is important, and covered with

a clean glass slide before clamping in the holder. A similarly prepared cell containing magnesium carbonate is used as a reference standard.

Light scattered by the sample passes the baffles, *J*, and falls on a glass filter held in a rotating stainless steel holder, *E*, fitted in a light-tight manner into a slot in an aluminium block. Stainless steel was found to produce less friction and stand up better to the wear imposed on this member than an aluminium holder. Following the selective filters is a cell containing a 1% copper sulphate solution. This cell is identical with the water cell except that it has an ordinary screw cap since openings for water circulation are unnecessary. The iris diaphragm, *G*, is placed immediately in front of the photo-cell, *H*, and is adjustable by a short lever, *I*, at the side of the instrument. The centre inner surface between the sample holders and the photo-cell must be light-tight and finished with a flat black paint.

## Accessory Equipment

The following accessory parts, obtainable from the stated sources, have been found suitable after considerable experimentation.

*Plexiglass cells*—made to dimensions and specifications by Stricker-Brunhuber Corp., New York, New York.

*Selective filters*—

Blue (400–500) Jena BG12, or Corning Signal Blue No. 556.

Green (500–600) Wratten W58, or a combination of Corning Light Theatre Blue No. 502 and H. R. Noviol No. 352.

Red (600–750) Jena OG3 or Corning H. R. Lantern Red No. 244.

*Iris diaphragm*—Leitz catalogue No. 8503.

*Photo-cell*—Visitron F-2A. G.-M. Laboratories Inc., Chicago.

*Galvanometer*—Box type Catalogue No. 4625, Rubicon Co., Philadelphia.

*Transformers*—The Variac transformer, General Radio Co., Cambridge, Mass., used for controlling the light source is generally unnecessary if an iris diaphragm is used, but may be required in special circumstances. Where line voltage is subject to some variation a constant voltage transformer will improve the accuracy and facilitate rapid operation. Suitable types are available from the Sola Electric Co., Chicago.

## Operation

The manipulation of the new machine is simple and rapid. The filter holder is rotated until the desired filter is in position, the standard white holder swung into place, and the galvanometer deflection adjusted to the desired value with the diaphragm. The standard white holder is then swung out, the test sample swung into position and the galvanometer deflection observed. This sequence of operations can be performed in less than a minute for each colour filter used. In fact, with reasonably constant voltage, the three colour components can be measured in two minutes, including the

time required to place the test piece in the holder and remove it. This is a much more rapid rate than attainable with the older instrument and greatly facilitates the measurement of colour in routine operations such as process control. The apparatus is not only suitable for measuring the colour of such solids as meat, but has also been applied to ground materials, such as flour.

## Accuracy

The accuracy of the new model can be compared with that of the old model, or with the error of sampling such biological materials as meat, from the results presented in Table I. The mean values and the standard errors of a single measurement were computed from duplicate measurements on some 30 to 40 samples picked at random from routine observations. The measurements on bacon with the old and new models were not made on the same samples and this may account for a portion of the difference between the mean values obtained with the two instruments. The greater part of this difference, however, is attributable to the leakage of light in the older model. The "dark" constant obtained with a standard black, as a test sample, was about 22% of that obtained from the standard white in the earlier model, and only 4% in the new model. This reduction in the dark constant was brought about largely by the insertion of the baffle plates, *J*, in the new instrument. The difference of 18% accounts for most of the difference between the corresponding means reported in Table I.

TABLE I

PRECISION OF NEW COLOUR COMPARATOR AS COMPARED WITH THAT OF OLDER INSTRUMENT AND SAMPLING ERROR

| Colour component | Old instrument | | New instrument | | | | |
|---|---|---|---|---|---|---|---|
| | Bacon only | | Bacon | | Pork | | |
| | Mean | Std. error (instrument) | Mean | Std. error (instrument) | Mean | Std. error (sampling) | Std. error (instrument) |
| | | | | Initial colour | | | |
| Red | 43.2 | 1.41 | 25.4 | 1.00 | 25.7 | 1.48 | 1.13 |
| Green | 31.1 | 0.87 | 14.5 | 0.45 | 16.2 | 1.12 | 0.77 |
| Blue | 28.3 | 0.61 | 11.5 | 0.36 | 11.9 | 0.74 | 0.64 |
| Brightness | | | | | 53.9 | 3.37 | 2.29 |
| | | | | Colour stability* | | | |
| Red | 5.35 | 1.30 | 4.03 | 0.60 | 2.19 | 0.43 | 1.04 |
| Green | 3.66 | 1.03 | 2.70 | 0.50 | 0.77 | 0.53 | 0.92 |
| Blue | 2.57 | 0.89 | 2.50 | 0.40 | 0.87 | 0.37 | 0.63 |
| Brightness | | | | | 3.83 | 1.33 | 2.22 |

**Mean change of scatter, usually a decrease from the initial value.*

The standard error of the instrument was computed by statistical methods from the difference between duplicate observations. The two values were obtained by cutting a piece of meat and making a single measurement on each of the two surfaces so produced. This practice tends to exaggerate the instrumental error by including the error of subsampling the test surface, and duplicate measurements on the same surface can be checked within narrower limits. The results of measurements of both the initial colour and colour stability of bacon show that the standard error of the new instrument is about half of that observed on the old instrument.

When the standard error of the initial colour measurement is expressed as a percentage of the mean for the corresponding colour component, the accuracy varies from about $\pm 2$ to 4%. This may appear to represent satisfactory precision, but it must be remembered that a difference of 10 to 20% in scatter represents the difference between a satisfactory and wholly unacceptable colour as judged by visual standards. In these circumstances it might appear that a still higher instrumental precision would be desirable for making fine distinctions, without the need for tedious replication.

In connection with an investigation into the storage of pork, provision was made for estimating both the sampling and instrumental errors by making duplicate measurements on duplicate pieces of pork taken from the same part of the same carcass and treated identically throughout. An analysis of variance showed that the combined sampling and instrumental errors were significantly greater than the instrumental error alone. It appears therefore that the sampling error is the source of variation limiting the accuracy of initial colour measurements and that little, if any, increase in precision would be accomplished by further refinements in the instrument.

These results were analyzed further with the object of determining the method of measurement capable of yielding the greatest precision for a limited number of observations. The last two columns show the standard error of sampling and instrumental measurements independently, on a single observation basis. The sampling error for the initial colour estimations is in all instances larger than the instrumental error. It is therefore evident that four single instrumental observations on four separate samples taken from the test material would yield a more accurate estimate of the true colour than duplicate instrumental readings on two samples. In other words, the instrumental error is not the factor limiting the accuracy of colour measurements on meat.

A somewhat different situation exists with respect to the error of estimating the change in colour during colour stability measurements (lower section, Table I). Although the standard instrumental error is of the same order as that for the initial colour measurement, the change in colour scatter is much smaller than the original scatter and is consequently estimated with much less precision on a percentage basis. As can be seen from Table I, the sampling error of colour stability measurements is less than the instru-

mental error. In these circumstances precise estimates of colour stability can be obtained with the present equipment only by adequate replication.

From the standpoint of practical colour measurements on a particular material, meat, flour, etc., the most important factor is to select colour filters that will yield the maximum information on colour quality for the material in question. In this respect the new instrument is the same as the old, since the filters separating the three broad colour bands are the same. Spectroscopic studies of meat are now under way with the object of determining the practicability of using more selective filters. Should such a modification be desirable, it is possible that the use of extremely narrow colour bands might require more sensitive measuring equipment.

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# STUDIES ON *OESTRUS OVIS* L.[1]

By A. Murray Fallis[2]

## Abstract

The incidence of infection of *Oestrus ovis* larvae in lambs has been determined. Flies were obtained from larvae which pupated in the laboratory. The three larval stages of the fly were studied and characteristic features illustrated. Information relative to the rate of growth of the larvae was obtained from a routine examination of infected animals and experimental infection of lambs. The effect of the parasites on these experimentally infected animals was noted.

## Introduction

The investigation was concerned with a study of the habits and development of the parasitic stages of *Oestrus ovis*. The incidence of infection was determined from an examination of sheep and lambs, one year old and under, received at a local abattoir from scattered points in Ontario and Western Canada. The rate of development was studied by the routine examination of slaughtered animals throughout the year and by experimental infection of parasite-free lambs.

## Incidence of Infection

Of 698 animals examined, 50% were infected with larval stages of the parasite. A higher incidence would be obtained if the total included only the older animals, for over 90% of the lambs examined from August to May were infected.

## Length of Life of Flies

Flies (Fig. 1) were reared from larvae which pupated in sand in the laboratory. Twelve flies were obtained in this way between May 2 and September 24, which are the first and last dates of emergence. Eight of the flies kept at room temperature lived an average of 16 days, one living for 28 days and two for 10 days only. This may be significant, for if flies remain alive and active for two weeks in their natural environment, a relatively small population might be capable of producing a high incidence of infection.

Mitchell and Cobbett (2) demonstrated that small, first-stage larvae could be expelled from female flies by pressing their abdomens. Hadwen has informed the writer that he used the same method to obtain larvae of a related fly, *Cephenomyia nasalis*. It seems likely, therefore, that *Oestrus ovis* is viviparous although Seguy (5) claims it is oviparous as well.

## Larval Stages

The smallest larvae found measured 0.8 to 1.0 mm. in length following fixation in hot alcohol, whereas the largest measured 24 mm. The former were taken on September 2 and November 25 respectively, the latter on

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*Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Ont.*

[2] *Research Fellow.*

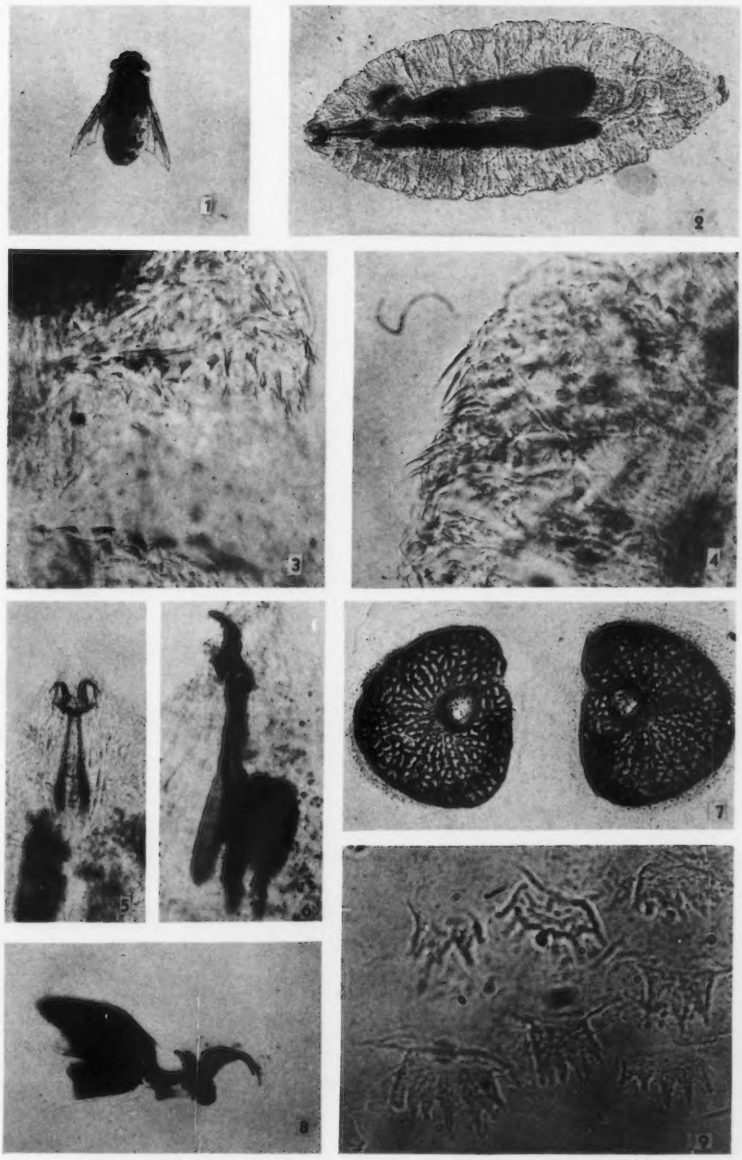
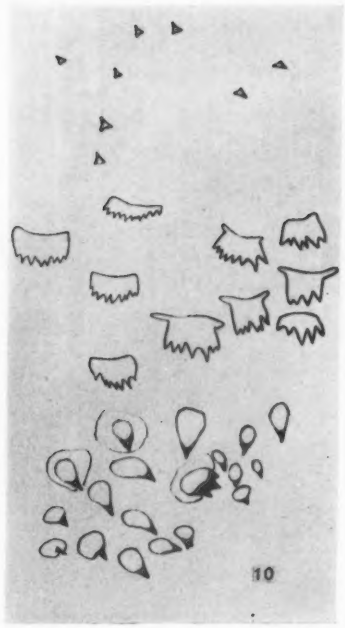


FIG. 1. *Adult fly, Oestrus ovis.* × 1½.
FIG. 2. *Ventral view of first instar larva. Note row of curved spines at posterior.* × 40.
FIG. 3. *Typical spines on ventral surface of segments of first-stage larva* × 300.
FIG. 4. *Typical spines at ventro-lateral margins of segments of first-stage. larva.* × 300.
FIG. 5. *Ventral view of pharyngeal sclerites and oral hooks of first-stage larva.* × 80.
FIG. 6. *Lateral view of pharyngeal sclerites and oral hooks of first-stage larva.* × 400.
FIG. 7. *Posterior spiracles of second-stage larva.* × 80.
FIG. 8. *Lateral view of pharyngeal sclerites and oral hooks of second-stage larva.* × 40.
FIG. 9. *Typical spinous plates on the ventral surface of the middle segments of second-stage arva.* × 400.

*All the photographs were made from untouched negatives.*









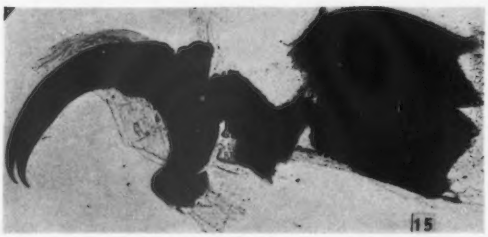





FIG. 10. *Outline drawings of typical spines on ventral surface of second-stage larva. Those on the anterior segment are shown in the upper part of the figure, those on Segments 2 to 10 in the middle part of the diagram, and those on Segment 11 in the lower part of the figure.* × 400.

FIG. 11. *Photomicrograph of typical spines on ventral surface of 11th segment of second-stage larva.* × 400.

FIG. 12. *Dorsal view of third-stage larva.* × 1½.

FIG. 13. *Ventral view of third-stage larva.* × 1½.

FIG. 14. *Typical spines on ventral surface of third-stage larva. There are eleven rows of these.* × 40.

FIG. 15. *Lateral view of pharnygeal sclerites and oral hooks of third-stage larva.* × 40.

FIG. 16. *Lateral view of pupa.* × 1½.

FIG. 17. *Ventral view of pupa.* × 1½.

FIG. 18. *Dorsal view of pupa.* × 1¼.

*All the photographs were made from untouched negatives.*

March 16. First-stage larvae (Fig. 2) attained a maximum length of 5.0 mm. Characteristic features of this instar are the transverse rows of spines on the ventral surface of the segments (Fig. 3), the longer spines on the latero-ventral margins (Fig. 4), the row of curved spines on the posterior segments (Fig. 2), and the size and structure of the pharyngeal sclerites (Figs. 5 and 6).

Second-stage larvae measured 3.0 to 14.5 mm. The posterior spiracles of this stage are very apparent (Fig. 7). The pharyngeal sclerites and oral hooks are larger and have undergone some change in form (Fig. 8) from those of the previous stage. According to Portchinsky (3), quoted by Rodhain and Bequaert (4), the first-stage larvae, after penetrating to the sinuses, lose their spines and change to the second-stage. This he considers is a state of rest in which they pass the winter. It is evident from the present studies, however, that winter may be passed in the first-stage as well. Moreover, second-stage larvae are not devoid of spines (Figs. 9, 10, and 11), although they differ from those on first instar larvae. The most anterior and posterior rows of spines have a single spinous process (Figs. 10 and 11), whereas those on the intervening segments have the form of small plates terminating in a serrated edge (Figs. 9 and 10).

Third-stage larvae measured 9.5 to 24.0 mm. in length (Figs. 12 and 13). The eleven rows of spines on the ventral surface (Fig. 14) are prominent and all have a similar shape. The posterior spiracles are larger and more heavily chitinized than those of the second instar larvae. The pharyngeal sclerites are also larger and show further modification (Fig. 15).

Many of the lambs examined were harbouring all three stages of the parasite. This confirms an observation made almost 150 years ago by Clark (1) who wrote ". . . quite young and full-grown larvae may be found in the sinuses at the same time."

## Rate of Development of Larvae

The rate of development of the larvae varies within wide limits. Spring lambs in Ontario are probably seldom exposed to the parasite before the beginning of May. No flies were taken in the field at this time but one was reared in the laboratory on May 2. On July 29 a larva, which formed a pupa from which a fly emerged, was recovered from the sinus of a lamb autopsied on this date. Thus it would seem the parasite cycle in spring lambs may require about three months in early summer. This confirms the work of Mitchell and Cobbett (2) who found the complete development of the parasitic stage in spring lambs in Texas and New Mexico required $2\frac{1}{2}$ to $3\frac{1}{2}$ months.

A study of natural as well as artificial infections indicates that development may not always be so rapid. This was shown by the presence of larvae less than 2 mm. long in the lambs examined throughout the fall and winter months when the temperature was such as to preclude the possibility of recent infection. On September 16, for example, 97% of 46 larvae obtained from five lambs examined were less than 5 mm. in length. Excluding one larva

which was 12 mm. long, the average length for the remaining 45 was 1.9 mm. On December 16, if we exclude one larva of 7.5 mm. from 75 obtained from five animals, the average for the remainder, which were all less than 3 mm., was 1.8 mm. On March 16, 43 larvae were recovered from two lambs. Three of these measured 15.3, 7.8, and 5.6 mm., respectively. The remainder were all less than 4 mm. in length, the average being 2.2 mm. A summary of the infection found in lambs at different times of the year is given in Table I.

TABLE I

| — | Total number of larvae | Percentage of larvae in different size groups | | | |
|---|---|---|---|---|---|
| | | 0–5 mm. | 6–10 mm. | 11–15 mm. | Over 15 mm. |
| March | 74 | 10.8 | 46.0 | 10.8 | 32.4 |
| April | 81 | 60.5 | 14.6 | 12.2 | 12.2 |
| May | 62 | 43.5 | 24.2 | 22.6 | 9.7 |
| June | | No parasites found in 50 spring lambs examined | | | |
| July | 93 | 75.4 | 7.5 | 14.0 | 3.2 |
| August | 275 | 77.1 | 4.0 | 9.1 | 9.8 |
| September | 169 | 100.0− | + | 0 | 0 |
| October | 89 | 97.5 | 2.5 | 0 | 0 |
| November | 380 | 98.6 | 1.4 | 0 | 0 |
| December | 209 | 98.5 | 1.5 | 0 | 0 |
| January | 62 | 96.7 | 3.3 | 0 | 0 |
| February | 62 | 96.7 | 3.3 | 0 | 0 |
| March | 93 | 94.6 | 3.2 | 1.7 | 1.7 |
| April | 64 | 78.0 | 7.8 | 11.7 | 3.0 |
| May | 50 | 54.0 | 16.0 | 20.0 | 10.0 |

The large number of first-stage larvae and the small number of third-stage larvae found from September to February is apparent. It is also seen that the number of second- and third-stage larvae recovered is much smaller than the number of first-stage larvae. The majority of the small larvae are found in the nares and many of them are probably sneezed out, whereas the larger larvae escape as they are usually found in the sinuses. Many first-stage larvae would be overlooked if the sinuses only were examined.

It was thought that the environmental temperature of the host might affect the rate of growth of the larvae. Three infected lambs were moved from an outdoor, midwinter temperature to a heated building, other infected lambs were left outdoors. One of the three lambs was left as a control. The second was given additional infection by transferring larvae less than 2 mm. long into its nostrils the same day as they were removed from infected animals. The third was given additional infection weekly by transferring small larvae to it. The first two lambs were slaughtered five weeks after being brought indoors and the third five weeks later. Control animals from outside were examined at the same time. The results are given in Table II.

An additional experiment was carried out in which two spring lambs, one month old, were infected by transferring larvae less than 2 mm. long to their nostrils. The lambs were kept in a heated building. Lamb No. 1 died

TABLE II

| — | Time between infection and slaughter, in weeks | Number of larvae recovered | Maximum size, mm. | Minimum size, mm. | Average size, mm. |
|---|---|---|---|---|---|
| Lamb No. 1 (control) | 5 | 5 | 2.5 | 2.2 | 2.3 |
| Lamb No. 2 | 5 | 8 | 2.5 | 2.2 | 2.3 |
| Outdoor control to No. 2 | ? | 26 | 7.5 | 1.9 | 2.9 |
| Lamb No. 3 | 10 | 50 | 18.0 | 1.5 | 5.0 |
| Outdoor control to No. 3 | ? | 15 | 5.6 | 1.9 | 2.5 |
| Outdoor control to No. 3 | ? | 28 | 15.3 | 1.8 | 2.4 |

of pneumonia eight weeks after being infected. It was autopsied, but not immediately following death so that some larvae may have escaped from it. Lamb No. 2 was autopsied five weeks after being infected. The results are indicated in Table III.

TABLE III

| — | Time between infection and slaughter, weeks | Number of larvae recovered | Maximum size, mm. | Minimum size, mm. | Average size, mm. |
|---|---|---|---|---|---|
| Lamb No. 1 | 8 | 10 | 7.7 | 2.1 | 4.9 |
| Outdoor control to lamb No. 1 | ? | 17 | 21.0 | 1.9 | 11.1 |
| Lamb No. 2 | 5 | 14 | 17.0 | 1.8 | 6.8 |
| Outdoor control to lamb No. 2 | ? | 27 | 17.0 | 2.0 | 7.0 |

It appears from the above-described experiments, therefore, that an increase in the temperature of the host's environment does not necessarily cause an increment in the rate of growth of the larvae. The rate of growth would seem to be variable at different times of the year. All the larvae used in the above experiments were several weeks old when transferred to the experimental animals and yet when these lambs were slaughtered several weeks later small larvae were still present. On the other hand, it was seen from the routine examination of spring lambs in early summer that the parasites reach maturity in the course of 2 to 3 months.

It also appears from these studies that there may be at least two generations of the fly per year in this country. In view of this, it is surprising that Rodhain and Bequaert (4) report only one generation of adult flies per year in Europe. They also point out the cycle is more rapid in Africa and that sheep are probably infected at all times since they always found second as well as third-stage larvae in all the animals examined. In the present study 63% of the infected animals harboured larvae in two or more instars. Most of the animals which harboured one stage only were examined during the

winter months. This raises the question as to whether or not there are more than two generations of the fly per year in Africa.

## Pupa

Pupae (Figs. 16, 17, and 18) were obtained by leaving mature larvae in dry sand at room temperature (approximately 20° C.). Metamorphosis was not successful in all pupae. The first larva which pupated was taken on April 5 and the last on August 12. The pupal period varied from 19 to 34 days with an average of 29 days in 12 specimens.

## Effect of Larvae on Host

The larvae caused a definite irritation to young animals. This was especially apparent in those which were experimentally infected. They sneezed and rubbed their noses on the wall. The "snuffing" was continued throughout life but was not so marked after the first few days. About two weeks following infection there was a slight discharge of mucus from the nostril of each lamb. At the time of autopsy the sinuses of both lambs were partially filled with purulent material. The pus contained large numbers of eosinophile leucocytes. Large numbers of these cells were also apparent in the mucosa of one of the lambs. First-stage larvae were frequently found with red blood cells in their intestines, but, as it was only found in those that had been bathed in blood, it is not known whether they were ingested by the parasite from blood present in the nasal cavities following the slaughter of the animal.

## Acknowledgments

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